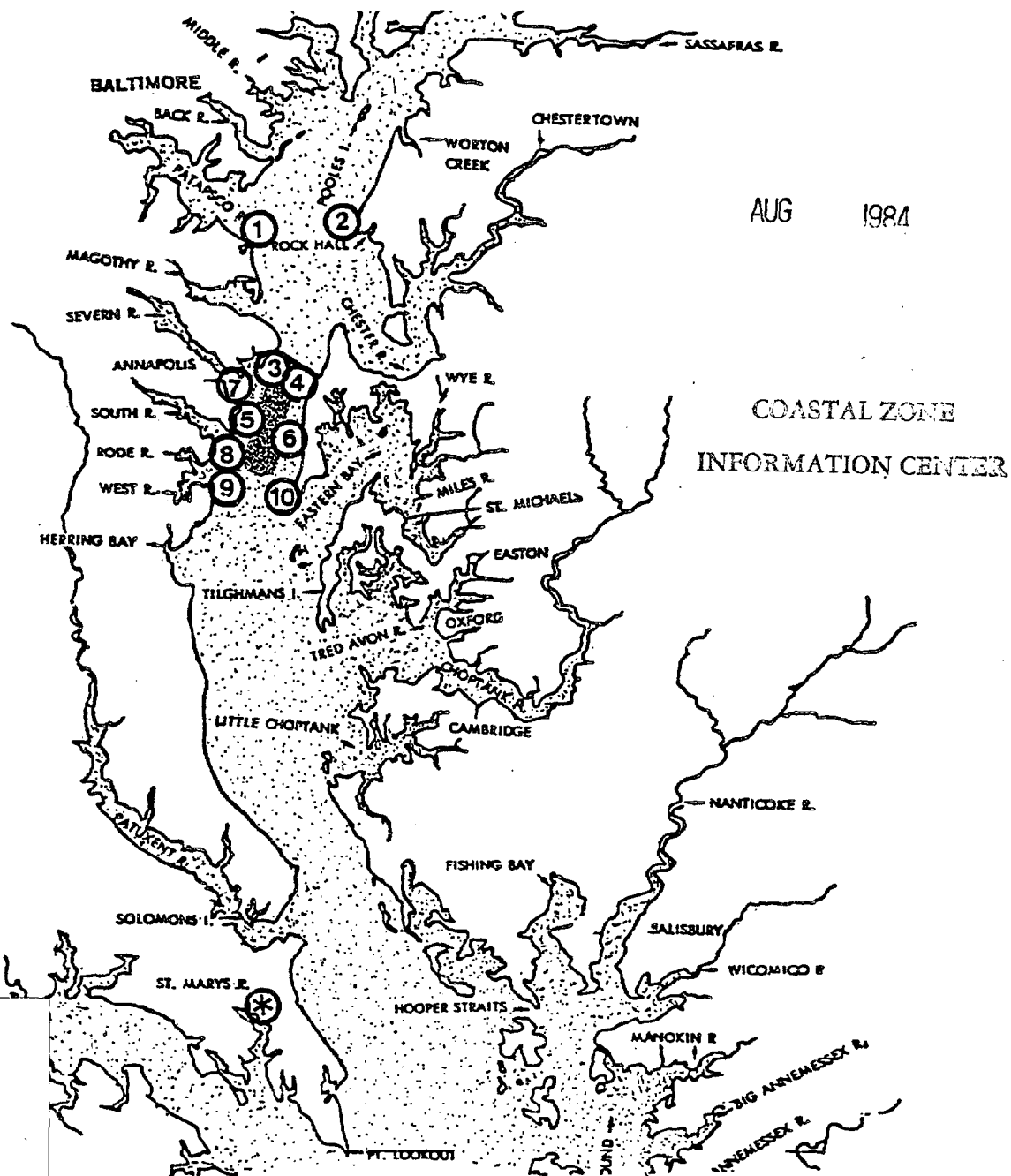


PETROLEUM HYDROCARBONS IN THE ANNAPOLIS ROADS AREA OF THE CHESAPEAKE BAY

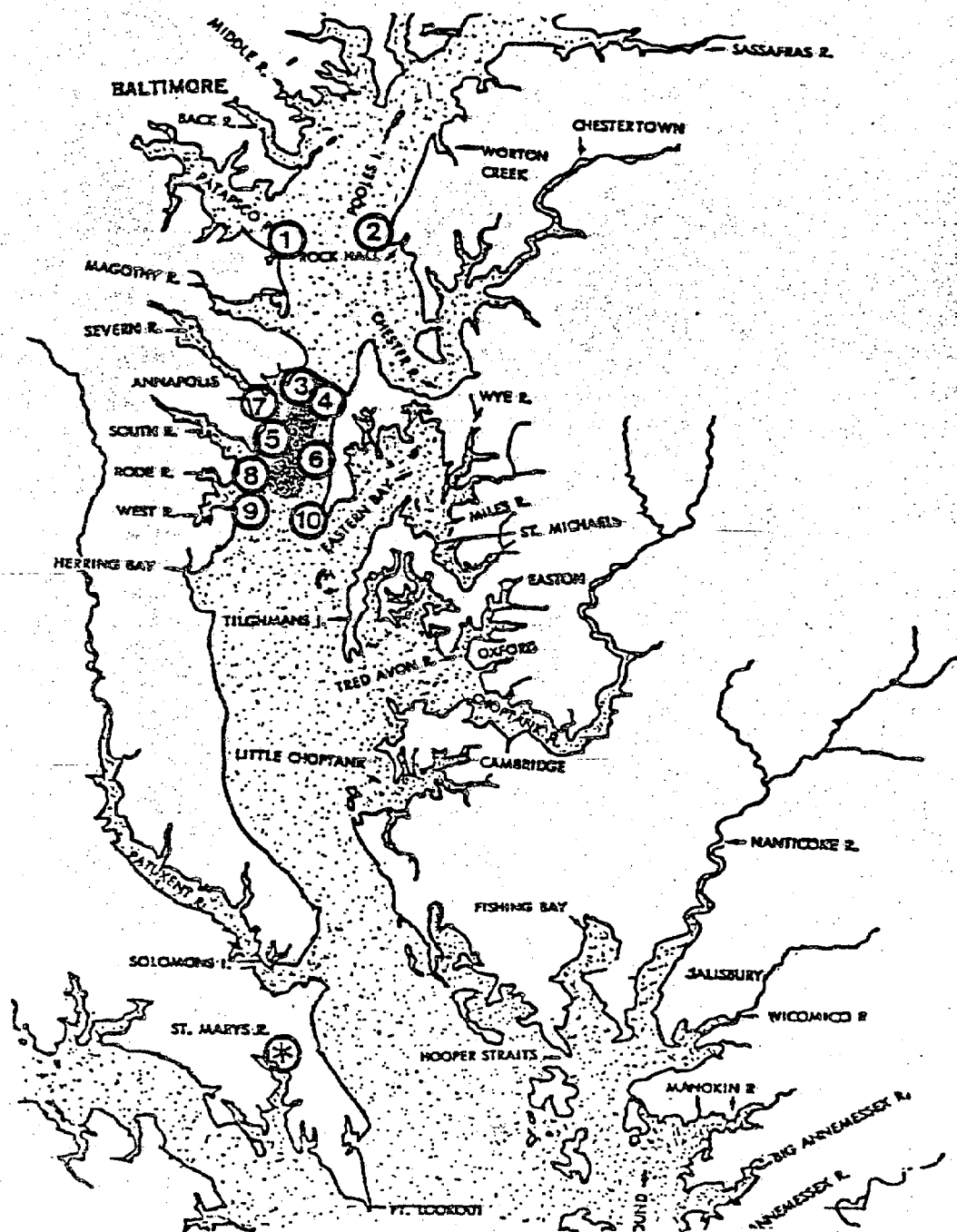
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PETROLEUM HYDROCARBONS IN THE ANNAPOLIS ROADS AREA OF THE CHESAPEAKE BAY



Hatch, Walter I.

Hydrocarbon levels in the Annapolis Roads area
of the Chesapeake Bay as determined through
Analysis of whole body and gill tissues of the
American Oyster (*Crassostrea virginica*)

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FINAL REPORT

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EXECUTIVE SUMMARY:

The baseline sampling program demonstrates that the mid-region of the Bay has a persistent background of petroleum hydrocarbons. The highest levels are at the Patapsco Station and reflect anthropogenic inputs from heavily industrialized and densely populated areas. Most samples of both biomonitors and sediments from this region show both chronic and recent inputs as indicated by a series of resolved peaks over a background of degraded petroleum hydrocarbons. The distribution of components present indicate a mixture of sources. It is against this background of chronic inputs that any additional inputs must be measured.

The analysis of oyster gill tissues demonstrates that the gills of these biomonitors reflect day to day changes in both the quality and quantity of petroleum hydrocarbons present, as they both accumulate and depurate hydrocarbons rapidly.

Comparison of the petroleum hydrocarbon content of the whole body tissue to that of gill tissues alone shows that the whole body tissue integrates the varying levels of oil present, and therefore represents a measurement of the mean content of oil in the water column whereas the gills indicate short-term fluctuation. The many resolved peaks, including many low boiling compounds, and the high concentrations observed in some of the gill monitors, indicate recent discharges into the Roads area. Each spill event had a unique chromatogram (fingerprint) and could therefore be traced to its source. Thus, this study has shown that oyster gill tissues can be used as a short-term monitor for discharges of oil into the Roads area. Gill monitors could furthermore be used equally well to monitor short term-inputs from non-point runoff following rainstorms or for continuous monitoring of oil handling facilities.

The levels of petrogenic hydrocarbons indicated by biomonitors in the upper Bay are elevated and comparable to those found in other urbanized industrial areas. The hydrocarbon levels in the Annapolis anchorage are lower than those in the upper Bay and suggest a small but measurable contribution from the area of the anchorage. The finite rate of exchange of bay water with coastal ocean water prevents the degree of dilution found around other coastal harbors and suggests that the upper Bay would suffer greater impact than would a coastal harbor with the same petrogenic inputs.

The levels of hydrocarbons in both the upper Bay and the anchorage are below that which would result in obvious biological effects. Recent evidence, however, suggests that subtle sublethal effects on bivalve molluscs would result from exposure to the concentrations found in the upper Bay and at some locations within the anchorage.

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Annapolis Roads Study

INTRODUCTION

The area of the Chesapeake Bay immediately south of the Chesapeake Bay Bridge is used as an anchorage for vessels awaiting entrance to the Port of Baltimore. The number of vessels, principally colliers, waiting to anchor for servicing at the coal-loading facilities at the Port of Baltimore has increased dramatically since March 1980 (Fig. 1) (Ostrum and Anthony, 1982). In addition, the length of stay of colliers in Annapolis Roads has increased from a previously normal level of around five days to a high of two months (Fig. 2) (Ostrum and Anthony, 1982). Anywhere from less than a dozen to more than three dozen ships may be anchored in this "Annapolis Roads (Anchorage)" area at one time. The Annapolis Roads will continue to be used for a minimum of two to three years during the continued development of facilities at the Port of Baltimore.

Recently, public concern has been voiced with regard to the potential for oil releases from vessels utilizing the Annapolis Roads. Although bilge pumping should not occur as these ships have holding tank capacity, concern arises with respect to the potential for accidental releases. Spills of oil from unidentified sources have occurred in the Roads area, and some have impacted limited areas of the Eastern Shore.

As the number of ships and their length of stay in the Annapolis Anchorage has increased, concern has been expressed that the opportunity for illegal discharges of fuel or oily wastes, either by accident or through neglect, may also increase. Attempts have been made to analyze the available information to determine if illegal discharges occur, if they are increasing, and if water quality impacts from such discharges were measured. The impact of these wastes on the aesthetic qualities of the water and shoreline were also of concern (Ostrum and Anthony, 1982). There were, however, several complicating

factors which made such an analysis difficult and inconclusive. For example:

- There is very little quantitative data available on spill events and water quality within the Anchorage.

- While reports of spill events or illegal discharges observed in the Anchorage can be counted, their accuracy in reflecting actual illegal discharges occurring from ships in the Anchorage is uncertain since:

- Ships are commonly discharging substances such as ballast, cooling water, and treated sewage which can be mistaken for illegal discharges.

- When slicks of other foreign substances are observed in the water it is not always possible to determine their origin, or what they are composed of. For example, slicks formed by organic matter are commonly associated with tidal fronts.

- Illegal discharges may not be observed (especially if they occur at night) or if observed, may not be reported.

- Because the backlog of ships is so visually obvious, enforcement officials and others who use the water probably exhibit a greater awareness and concern over pollutant discharges from ships. Thus, an increase in the number of reported pollution incidents could be explained by increased monitoring, as well as by an increase in the number of spill incidents. On the other hand, since ship masters are aware of the increased monitoring, intentional dumping may be more carefully disguised, thus less frequently detected.

It is believed, however, that most masters respond to increased monitoring by adhering to the existing laws.

- Even when evidence for illegal discharges does exist (e.g. oil slicks, or garbage with foreign labels), it is difficult to determine

if the wastes emanated from a ship at anchor. The Anchorage is located in close proximity to the major shipping channel into Baltimore which is frequented not only by passing oceangoing vessels, but also by coastal traffic, consisting of tugs, barges, coasters, and similar craft (from Ostrum and Anthony, 1982).

Notwithstanding the difficulties cited above in interpreting the available information on illegal discharges, there is evidence that discharges of wastes from ships in the Anchorage have occurred (Ostrum and Anthony, 1982). In two oil spill cases, chemical evidence was collected that suggests that oil from the spills matches the oil in samples taken from the ships involved; however, this evidence has not yet been established in a court of law (Ostrum and Anthony, 1982).

In order to complement the Office of Environmental Programs studies, which were primarily concerned with sewage inputs, this study purports to investigate the distribution and concentration of petroleum hydrocarbons in the mid-Chesapeake Bay in general, and the Annapolis Anchorage area in particular. This two phase study provides background information on the levels of hydrocarbons currently existing in the Roads and on the identification of point inputs into this area.

Most sea water concentrations of petroleum hydrocarbons range from parts per trillion (10^{-12} g/g) to parts per billion (10^{-9} g/g). Although methodologies for the measurements of pollutants at these low levels in sea water are being developed, they are not yet in routine use (Goldberg et al., 1978). The ability of bivalve mollusks to concentrate hydrocarbons over levels in the environment circumvents the necessity to obtain large volumes of water for extraction and analysis (Ehrhart, 1972; Stegeman and Teal, 1973; Burns and Smith, 1977; Goldberg et al., 1978). We chose the American oyster, *Crassostrea virginica*, as our monitoring organism because it readily concentrates hydrocarbons in its tissues, it is indigenous and

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common in the study area, and is an economically important species. Stegeman and Teal (1973) demonstrated uptake of petroleum hydrocarbons in oysters is related to lipid content of the organisms, and that the rate of uptake was linear from 0-450 $\mu\text{g}/\ell$, greatly exceeding the range likely to be experienced in the proposed study.

Uptake of hydrocarbons can be viewed as an equilibration process controlled by the relative solubilities of various classes of hydrocarbons in water compared to lipids (Hamelink et al., 1971). Since uptake and discharge processes proceed simultaneously, the net amount retained by the organisms is related to the saturation of its lipid stores and concentrations in the water. The equilibration magnification factor calculated for oysters (Stegeman and Teal, 1973) is approximately 3×10^5 . Fossato and Canzonier (1976) reported similar magnification factors in *Mytilus* (mussels) experimentally dosed with diesel oil. Thus, the results of a bivalve analysis taken from an environment allows an estimate of the minimum amount to which the animal was exposed in the water column. If the concentration of petroleum hydrocarbons is below the maximum saturation level of the organism (most certainly the case in the proposed study), the lipid magnification factor can be used to back-calculate the concentration of hydrocarbons in the water column. Burns and Smith (1977; 1981) showed that the petroleum hydrocarbon level in water samples was close to those predicted by back-calculations from mussel tissue levels.

Therefore, in areas subject to chronic inputs of petroleum, bivalve tissue analysis provides a means of identifying input sources, types of hydrocarbons present, and integrated average concentrations of petroleum hydrocarbons in the water column (Farrington and Quinn, 1973; Ehrhardt and Heinemann, 1975; Farrington et al., 1976; Burns and Smith, 1977). Values obtained by bivalve analysis provide an integrated sample over more time

than can be measured with an intermittent water sampling program (DiSalvo et al., 1975; Fossato and Canzonier, 1976). Also, results of bivalve analyses are easier to interpret than water samples with low level petroleum contamination since bivalves tend to show less interference from waterbourne biogenic plant hydrocarbons. Bivalve analyses, therefore, provide a clearer picture of average water column hydrocarbon concentration as long as the results are interpreted with the biological mechanisms of uptakes, retention, and depuration in mind.

In addition, a review of the literature indicates that bivalves are fairly resistant to chemical stress and that many other marine and estuarine organisms are more sensitive (Moore et al., 1975; Hyland and Schneider, 1976). Therefore, if bivalves such as oysters show physiological stress from petroleum levels present in the environment, we can safely predict that the ecosystem as a whole is stressed. Fossato and Canzonier (1976) showed that petroleum hydrocarbons are rapidly accumulated by the gill tissue of bivalves taken from clean environments and placed in contaminated water. They found that the composition and concentrations of the oil hydrocarbons in the gills were similar to those in the water, but that it took several days for the hydrocarbons to be transferred from the gills to other parts of the body. Thus, the composition and concentration of oil hydrocarbons in gill tissue from oysters from a clean site, even if placed only briefly (1-2 days) in a contaminated area, will reflect the composition and ambient concentrations in the water at the contaminated site. This will be true even if the contaminated site has oil concentrations only slightly higher than those at the clean site.

It has not, however, been reported at this time whether the gill tissue from bivalves exposed for long periods to contaminated water will reflect the average petroleum hydrocarbon content of ambient water or the day to day concentrations. Thus, whole body petroleum hydrocarbon concentrations may

reflect integrated average oil hydrocarbon levels of ambient water, while the gill tissue may reflect day to day changes in these levels. It may be possible to assess both long-term average petroleum hydrocarbon concentrations of ambient water and short-term fluctuations in these concentrations utilizing one set of biomonitors, merely by analyzing whole body and gill tissue separately. The results of this evaluation have important consequences for the design of future short-term monitoring programs.

METHODS

Organisms:

The American oyster (*Crassostrea virginica*) was used as the biomonitor throughout the study. All oysters were collected from the relatively clean water of the upper St. Mary's River and maintained in our laboratory at St. Mary's College in flowing estuarine water (\bar{X} salinity 15%) subsequent to use.

Phase I: Background Petroleum Hydrocarbon Monitoring Stations:

To provide background information on the distribution and concentration of petroleum hydrocarbons in the mid-Chesapeake Bay and the Annapolis Roads in particular, a series of monitoring stations was established from the level of the mouth of the Patapsco River to the level of Bloody Point Bar (Table 1, Fig. 3). Two stations were located approximately 9 nautical miles below the Bay Bridge to provide background oil concentrations well downstream of the Roads area. The remaining six stations bracket Annapolis Roads and the two other potential major sources, the Severn and South Rivers.

Station 1: Downstream from Bell "27C" off Bodkin Neck at the mouth of the Patapsco River. This station was intended to monitor inputs from the Baltimore Harbor area, which would in part provide a source of background petroleum hydrocarbons for the Bay downstream of this location.

Station 2: Just downstream of 4 sec. Flash "14" off Swan Point, eastern shore. This station was intended to reflect the background of oil hydrocarbons entering the mid-Bay from further upstream.

Stations 3 and 4: These stations are located just downstream of the Chesapeake Bay Bridge and to the west and east of the shipping channel off Hacket Point and Stevensville, respectively. These two stations were

intended to reflect the background oil hydrocarbon concentrations just upstream of the Annapolis Roads area.

Stations 5 and 6: Just downstream of Bell "77" off Tolly Point, western shore, and just inshore of White Orange Nun "S" on Brickhouse Bar markers on the eastern shore, respectively. These two stations were intended to reflect oil hydrocarbon concentrations just downstream of the Annapolis Roads area.

Station 7: Just downstream of Fl "1" off Chinks Point at the mouth of the Severn River. This station was intended to reflect the oil hydrocarbon inputs to the Roads area from Annapolis Harbor area.

Station 8: Just upstream of Thomas Point Shoal Horn. This station was intended to reflect oil hydrocarbon inputs to the Roads area from the South River area.

Stations 9 and 10: Just west of Black/White Nun, N "32B", western shore, and Bloody Point Bar Horn, eastern shore, respectively. These two stations were intended to reflect the background petroleum hydrocarbon concentrations well downstream of the Roads area. These stations are relatively distant from any major point petroleum hydrocarbon sources and are in a hydrologically active region that is reasonably well mixed, providing comparison sites that probably reflect mid-Bay background conditions. At the time of the study, however, the Roads were occupied by vessels as far south as these stations.

Wire mesh bags containing twelve oysters each were set 0.5 M below the surface on taut-line buoy systems (Fig. 4) at each station. All monitors were left in place for the 19 day period from July 23, 1981 through August 11, 1981, at which time they were collected and frozen for subsequent analyses of whole oyster tissue and pallial fluids. Sediment samples were taken at each site with a stainless steel piston corer. The top 10 cm of each core

was frozen for subsequent analysis. All samples, both sediment and oysters, were stored frozen at -20°C in glass jars with aluminum foil caps, both of which were pre-rinsed with redistilled solvents. Temperatures and salinities at each station were recorded.

Phase II. Forty-eight hour gill monitors:

To assess the feasibility of using oyster gills as short-term bio-monitors, and to examine the short-term fluctuations in petroleum hydrocarbon concentrations around Annapolis Roads, a series of monitoring stations was established around the Roadstead from a point just below the Bay Bridge southward to Bloody Point Bar (Fig. 5, Table 2). Twelve oysters were placed in the mesh bags of the monitoring stations previously established.

The short-term monitors were initiated on August 13 and were reset on August 15 and 17, 1981. All short-term monitors were collected 48 hours after initiation and frozen for subsequent analysis of gill tissue only. In addition, an aliquot of the Bay baseline monitors collected on August 11 was included with the short-term monitors for gill analysis. The organisms were stored frozen at -20°C in glass jars with aluminum foil caps, both of which were prerinsed with redistilled solvents. Immediately prior to analysis of the gill monitors, the oysters were opened and the ctenidia (gills) were dissected free of the mantle and visceral mass while the latter was still frozen. All dissection was accomplished on a redistilled solvent-rinsed aluminum foil lined work surface using redistilled solvent rinsed implements.

Hydrocarbon Extraction and Lipid Content Determination:

Prior to analysis, 10 oysters from each monitoring station were washed, shucked while frozen, and the shells discarded. The soft tissues of 5 randomly selected oysters was then placed in one of two groups. One group

was refrozen in solvent-rinsed jars with aluminum foil lined caps and retained as a reference sample. The other group was analyzed for hydrocarbon content. Previous analyses using the procedures below have shown that a pooled sample of several individual bivalves provides a reasonable estimate of the mean hydrocarbon concentration (Krebs and Paul, 1980). The analytical group was homogenized with a Tekmar Tissuemizer^R at high speed for 4 minutes, producing a pooled tissue homogenate.

Percent dry weight was determined by pipeting 5 ml of each pooled homogenate into tared aluminum weighing pans and determining the net weight and then the dry weight after drying to a constant weight. A second five ml aliquot was weighed and extracted with three sequential 25 ml hexane washes. Extraction was accomplished by grinding the pooled tissue homogenate and hexane in a 50 ml centrifuge tube followed by centrifugation to separate the aqueous and hexane phases. The hexane phases were pooled, reduced to about one ml and quantitatively transferred to tared aluminum weighing pans for final evaporation of the hexane and determination of the total hexane extractable lipids.

A final 15 ml aliquot of the pooled tissue homogenate was weighed and prepared for GLC analysis according to the technique of Farrington, et al., 1981a. In this procedure 15 g of tissue homogenate were digested with 15 ml of 6N NaOH, mixed vigorously for 2 minutes, and then incubated at 30°C for 18 hours in a 50 ml Teflon-lined capped centrifuge tube. Following incubation, the saponified tissues were extracted with 20 ml of diethyl ether, and then three additional times with 10 ml ether washes using centrifugation to separate the aqueous and organic phases. The ether extracts were then combined and shaken occasionally in a 1000 ml flat-bottomed flask with 15-25 g of ignited anhydrous sodium sulfate and then let to stand overnight to remove residual water. The ether extracts were then transferred to

500 ml round bottom flasks and concentrated in a Roto-vap^R under reduced pressure. After transferral to 25 ml pear-shaped flasks, the extracts were further concentrated to less than 5 ml (2-5 ml depending on the lipid content). The concentrated extract was then loaded on a silica gel/alumina column and eluted first with hexane, then aliquots of 10 and 20% toluene in hexane. The hexane fraction is designated the F₁ fraction and contains the aliphatic hydrocarbons, while the toluene in hexane fractions are designated the F₂ fraction and contain the aromatic hydrocarbons. The F₁ and F₂ fractions were taken just to dryness using a Roto-vap^R, transferred to sample vials and stored at -20°C in one ml of hexane for subsequent analysis. Immediately prior to analysis, the F₁ and F₂ fractions were again taken just to dryness using a Mini-vap^R concentrator with dry, high purity Nitrogen and then re-dissolved in Chloroform for analysis.

Sediment Analysis: Each of the sediment samples was divided into two parts. The major portion of each sample was dried at 105°C for 24 hours and set aside for particle size and organic matter analysis. The remaining portion of about 25 g was retained for hydrocarbon analysis using a modification of the method of Farrington and Tripp (1975) which simultaneously extracts and saponifies the samples. The samples were transferred to tared pre-extracted cellulose thimbles and weighed. The sediments were extracted with 350 ml methanol for 48 hours in a Soxhlet apparatus. The apparatus was then cooled and 20 ml of toluene, 20 ml of 0.7 N KOH and 30 ml of glass distilled water were added. Refluxing was then continued for an additional 1.5 hours to saponify the sample. The hydrocarbons were then partitioned into hexane with three sequential washes in a separatory funnel. The combined hexane fractions were reduced to 5 ml in a Roto-vap^R and trickled over an activated copper column to remove sulfur compounds. The copper column was eluted with 50 ml of hexane and the elutant reduced to one ml for

column chromatographic fractionation into alkanes and aliphatics as were the oyster tissue extracts. These fractions were then analyzed with GLC as described below.

Gas Chromatographic Analysis: Both column chromatography fractions (F_1 and F_2) were analyzed on a six foot x 2 mm ID glass column packed with 1% Dexsil 300 on Supelcoport 80-100 mesh. The column was installed in a Hewlett Packard 5710A Gas Chromatograph equipped with dual film ionization detectors. Due to the very broad boiling range encountered in the analysis of heavy fuel oil components, a high injection port temperature (350°C) is required. This resulted in high bleed with long injection life from low temperature Thermogreen^R septa, or a short injection life with low bleed from high temperature Pyrosep^R septa. We overcame this difficulty by making a sandwich with the high temperature Pyrosep^R septum in contact with the hot injection port and the Thermogreen^R on top. This procedure resulted in low bleed septa with a highly extended injection life.

The chromatographic conditions were as follows: injection port and detector temperature 350°C , nitrogen carrier gas 40 ml/min, airflow 300 ml/min, hydrogen flow approximately 40 ml/min varied to optimize the detector for C_{28} normal alkane, injection volume 1 μl with a 0.5 μl solvent flush. A temperature programmed chromatographic run was utilized with the initial temperature set at 100°C , programmed to hold 2 min, and rise at 16° min to 330°C , holding for 8 min.

The resultant chromatograms were recorded simultaneously with a Hewlett Packard model strip chart recorder and a Hewlett Packard model 3391A reporting integrator. Fraction 1 compounds were quantified by the reporting integrator after calibration with an external standard containing 0.1 μg each of C_{12} through C_{36} , even carbon number normal alkanes. Fraction 2 compounds were quantified in the same way using 0.1 μg each of hexamethyl-

benzene, hexaethylbenzene, anthracene, pyrene, and benzanthracene as external standards. The mean response factor of all of the standards in each fraction was utilized to quantify any uncalibrated peaks.

The carbon preference index (CPI) was calculated from n-alkane abundances and was expressed as follows:

$$\text{CPI} = \frac{2 \times \sum n = C_{21} \text{ to } n = C_{31} \text{ (odds)}}{\sum n = C_{20} \text{ to } n = C_{30} + \sum n = C_{22} \text{ to } n = C_{32} \text{ (evens)}}$$

(Wehmiller and Lethern, 1975).

Quality Assurance:

Reagent blanks were run through the entire extraction and column chromatography process by substituting glass distilled water for the oyster tissue. The F_1 and F_2 fractions were concentrated and chromatographed under the same conditions as the samples.

Percent recovery was determined by spiking an aliquot of the pooled homogenates of control oysters. The spike consisted of nC_{12} - nC_{36} even carbon numbered alkane for F_1 recovery and hexamethylbenzene, hexaethylbenzene and anthracene for F_2 recovery.

Column chromatography separations were verified by running a standard containing C_{15} (N-pentadecane), C_{22} (n-docosane), androstane (a cyclic C_{19} saturated hydrocarbon), hexamethylbenzene (HMB), hexaethylbenzene (HEB), and anthracene (ANT, a multi-ring aromatic hydrocarbon).

RESULTS

Physical Parameters:

The temperature of the study site at initiation varied from a high of 26.5°C to a low of 25.0°C. The temperature within the Phase II short-term biomonitor study site did not vary measurably from 25.5°C. The salinities, as expected, showed more variation, from a low of 9.5% at Swan Point to a high of 15.5% at Bloody Point and Brickhouse Bars. Within the Phase II study site the salinity was less variable with a mean of 14.1% and a range of 12.5% to 15.5%. These physical parameters were well within the range of tolerance of the American oyster (*Crassostrea virginica*).

Station Recovery:

Of the stations set for the Phase I background monitoring program, all were recovered with the exception of Stations 13 and 14 at the east and west ends of the Chesapeake Bay Bridge. Station 15 at Tolley Point suffered damage resulting in the loss of most of the oysters, but sufficient tissue for analysis was recovered. The Phase II short-term monitors at the Bay Bridge were also lost each time they were set except for Station II4 on 8/13/81 and Station II3 on 8/15/81. The remainder of the stations were recovered with all organisms alive. Only one oyster death was recorded; at Swan Point (I3) on 8/11/81.

Quality Assurance:

Column Chromatographic Separation: Figure 3A-D illustrates that we achieved complete separation of the aliphatic hydrocarbons (C₁₅, C₂₂ and androstane, from the aliphatic masked aromatics (HMB, HEB) and multi-ring aromatic (ANT) into F₁ and F₂ fractions respectively. The F₁ fraction (Fig. 3B) contained greater than 99% of the aliphatic, while the F₂ fraction (Fig. 3C) contained 100% of the aromatics. The F₃ fraction (Fig. 3D) was

completely free of both F_1 and F_2 components. Percent recovery from the column chromatography procedures was $99 \pm 1.5\%$.

Solvent and Procedural Blanks: A representative chromatogram of a solvent blank is shown in figure 4A and demonstrates the lack of excessive septum bleed or baseline drift in the chromatographic process. A representative procedural blank is shown in Figure 4B. It should be noted that an unknown contaminate coeluting with $n\text{-C}_{28}$ is present in measurable concentrations.

Percent Recovery: The recoveries of standard hydrocarbons from spiked samples are presented in Tables 4A and 4B. The mean overall recovery of aliphatic hydrocarbons was 80.8% with a standard deviation of 16.0%. The mean recovery of aromatic hydrocarbons was 48.0% with a standard deviation of 17.2%. Both of these recovery percentages agree well with values reported in the literature (Farrington et al., 1982a).

Control Oysters:

Chromatograms of the "clean" oysters obtained from the St. Mary's River are shown in Figures 5A and B. The mean total aliphatic (F_1) hydrocarbon concentration is 9.47 ± 0.21 ug/g dry weight while the mean total aromatic (F_2) hydrocarbon concentration is 5.26 ± 0.10 . It should be noted that the odd numbered aliphatic hydrocarbons predominate in St. Mary's River oysters.

Phase I. Chesapeake Bay Background Hydrocarbon Levels:

Representative chromatograms of the aliphatic and aromatic hydrocarbons extracted from the bay background biomonitors are presented in Figures 7-10. The relative increase in the levels of even numbered aliphatics in relation to St. Mary's controls should be noted. The concentrations of aliphatic hydrocarbons (F_1) ranged from 6.4-49.6 ug/g dry weight with the highest values found in oysters from the upper bay. The concentrations of aromatic

hydrocarbons (F_2) ranged from 8.5 to 18.4 $\mu\text{g/g}$ dry weight with the highest value found at Chinks Point. The F_1 and F_2 hydrocarbon concentrations for the Phase I background biomonitors are summarized in Table IV.

There was considerable variation in both the total water and total lipid contents of oysters at different stations. Due to variation in the hydrocarbon concentrations when reported as $\mu\text{g/g}$ wet weight or $\mu\text{g/g}$ lipid, all data are reported on the basis of $\mu\text{g/g}$ dry weight.

Phase II. Short-Term 48 Hour Biomonitors:

The total hydrocarbon concentrations in gill tissue from the 48 hour short-term monitors are summarized in Table V. The concentration of aliphatics (F_1) varied from a low of 3.1 $\mu\text{g/g}$ dry weight, reflecting background levels, to a high of 197.7 $\mu\text{g/g}$ dry weight, most probably reflecting a spill incident. Measurements of duplicate subsamples of pooled homogenate are in good agreement, generally not varying by more than 15%.

To serve as references in the identification of the petroleum hydrocarbon "fingerprints" in the chromatograms of both the bay background and short-term biomonitors, fuel and crude oils were analyzed under the same chromatographic conditions. Typical chromatograms of whole (combined F_1 and F_2) Southern Louisiana crude oil, No. 6 fuel oil, and No. 2 fuel oil are shown in Fig. 19a, b, and c, respectively. Although 10 μg of each oil was injected, the concentrations reported by the reporting integrator were 1.3 μg for Louisiana crude, 3.5 μg for No. 2 fuel oil, and 1.4 μg for No. 6 fuel oil. As most of our samples show approximately equal concentrations of No. 2 and No. 6 fuel oils, the actual concentration of hydrocarbons reported here are most likely under-estimated by a factor of four (4).

Sediments

The concentration of hydrocarbons in sediments ranged from a high of 627 $\mu\text{g/g}$ dry weight at the Patapsco River station (Fig. 18A) to a low of 67 $\mu\text{g/g}$ at the Brickhouse Bar station (Fig. 18B). Sediment hydrocarbon concentrations at the other stations did not vary by more than a factor of two and averaged approximately 350 $\mu\text{g/g}$ dry weight. Chromatograms of all sediment samples, except the Brickhouse Bar station, showed a number of resolved, generally biogenic peaks over a fairly large UCM (Fig. 18A).

DISCUSSION

Station Recovery:

The low density and insolubility of petroleum hydrocarbons result in higher concentrations in surface waters at the time of a spill incident. Only over extended time periods are these compounds dispersed through the water column by wave action and vertical mixing. This necessitates maintaining biomonitors in surface waters in order to evaluate recent input activity, thereby creating problems with the recovery of these monitoring stations.

We have noted in other studies (Krebs and Paul, 1980; Hatch and Krebs, manuscript in preparation) that high station losses occur especially in the vicinity of crabbing activities. The current study further documents that there is a low probability of station recovery in areas of active crabbing. All of our monitoring stations were recovered with the exception of Stations 3 and 4 located at Hackett Point and Stevensville (Fig. 3, Table I,II), both areas of heavy crabbing activity. The situation is so severe in this area that on one occasion (8/15/81) a monitoring station was cut off within one hour of the time that it was set. It should be noted that all stations were clearly marked OIL POLLUTION STUDY and included our names and telephone number as required by the U. S. Coast Guard.

This problem is by no means restricted to the Chesapeake, prompting other investigators to set sub-surface floats equipped with radio transponders (Farrington, J., personal communication). Our own alternative proposal to utilize subsurface floats marked only with inconspicuous buoyant beer or soda cans failed to receive Coast Guard approval. Thus, with the exception of Bloody Point Bar (Fig. 1, Table II), we were unable to obtain data in the vicinity of crabbing activity.

Due to the chronic problem of station loss through either accidental or intentional cutting off of surface buoys, and to the potential hazard to navigation of unmarked shallow-set sub-surface floats, we recommend that subsequent surface water monitoring stations be marked with official Marine Police Buoys. We feel that this will greatly discourage vandalism and make the collection of heretofore unavailable data possible.

Quality Assurance:

From the chromatograms of the six component ($F_1 + F_2$) standard (Fig. 5A) and the F_1 (Fig. 5B), F_2 (Fig. 5C) and F_3 (Fig. 5D) fractions of this standard, it can be seen that our fraction separation protocol was effective. There is no measurable contamination of the F_1 aliphatic fraction by aromatics nor contamination of the F_2 aromatic fraction with aliphatic hydrocarbons. Even the difficult to separate aliphatic masked aromatics hexamethylbenzene and hexaethylbenzene were entirely confined to the F_2 aromatic fraction. The clean trace exhibited by the F_3 fraction (Fig. 5D) is indicative of near complete recovery of both the F_1 and F_2 fractions.

It can be seen from the chromatogram of a solvent blank (Fig. 6A) that there is no significant peak area contributed by either the injection syringe, solvent or the chromatographic system itself. The baseline rise and small peaks after $n\text{-C}_{28}$ are due to septum bleed. The procedural blank (Fig. 6B) however, demonstrates an unknown contaminant that coelutes with $n\text{-C}_{28}$. The source of this contaminant remains unidentified at present.

The percent recovery of hydrocarbon spikes shown in Table II are comparable with those reported in the literature (Farrington *et al.*, 1972b). The low recovery of low carbon number aliphatic and aromatic hydrocarbons is to be expected. These compounds have low boiling points and some losses

due to evaporation are inevitable, especially during those procedural steps in which the sample solvent is evaporated to near dryness. In addition, aromatic hydrocarbons in low concentrations may not completely elute from the cleanup columns used to separate the aromatics from the aliphatics.

Phase I: Bay Baseline Hydrocarbon Concentrations.

Petroleum hydrocarbons (oil) have become major contaminants of estuarine and coastal environments (Blumer, 1971; Wolfe, 1977; VanVleet and Quinn, 1978). Accidental spillages provide the largest transient inputs of oil, but the greatest contribution to the total inputs of anthropogenic hydrocarbons to these environments is from chronic inputs primarily of refined oils from land runoff, rivers, sewage discharges, industrial effluents, and oil refineries and terminals (VanVleet and Quinn, 1978; Read and Blackman, 1980). The magnitude of these petroleum contaminants is often sufficient to obscure the natural background of biogenic hydrocarbons produced by living organisms except in highly productive systems or during algal blooms (Erhardt and Heinemann, 1975). At present these pollutant sources are so ubiquitous that it is difficult to locate aquatic environments free from some degree of oil pollution (Farrington et al., 1976; Farrington et al., 1982a).

Hydrocarbons in estuarine environments, therefore, are derived from both petrogenic (oil) and biogenic sources. Since bivalves accumulate both biogenic and petrogenic hydrocarbons through their feeding and respiratory activities (Erhardt, 1972; Fassato and Siviero, 1974; Disalvo et al., 1975; Mix, 1979; Farrington et al., 1980; Farrington et al., 1982b; among others), even in "clean" environments they contain biogenic hydrocarbons over a background of generally degraded petrogenic hydrocarbons. Thus, it is not sufficient to merely quantify the amount of hydrocarbons present in

estuarine biota, but it is essential to distinguish the relative contribution of biogenic and petrogenic hydrocarbons to the total hydrocarbon concentration measured before the degree of pollution can be assessed.

Biogenic hydrocarbons produced by living organisms are characteristically a simple mixture of hydrocarbons in which odd carbon chain lengths predominate. Within the estuary, biogenic hydrocarbons are derived principally from algae (phytoplankton) containing the straight chain alkanes C_{15} , C_{17} , C_{19} , C_{21} (Clark and Blumer, 1967; Blumer *et al.*, 1971; Youngblood *et al.*, 1971; Shaw and Wiggs, 1980); algal and zooplankton detritus containing pristane (Avigon and Blumer, 1968; Blumer *et al.*, 1971) and marsh and terrigenous (land-derived) plant detritus containing odd carbon length alkanes C_{21} to C_{31} (Farrington and Meyers, 1975; Simoneit, 1977; Teal and Farrington, 1977; Boehm and Quinn, 1978).

Petroleum hydrocarbons, on the other hand, characteristically consist of very complex mixtures of both aliphatic (saturated) and aromatic hydrocarbons (Fig. 20). Gas chromatography (GLC) is a powerful analytical technique capable of separating and identifying many of the components of oil, and the analysis of any oil mixture produces characteristic traces (chromatograms) from which the oil mixture can be identified. Identification is normally accomplished by evaluating the characteristic pattern of chromatograms of the aliphatic hydrocarbons (the F_1 fraction) of oil samples. The aromatic hydrocarbons (the F_2 fraction) containing benzenes, naphthalenes, and other multiple benzene ring compounds, can also be useful in oil sample identification, but require the use of sophisticated and expensive gas chromatography-mass spectrophotometry (GC-MS) analysis for positive identification of individual components. Chromatograms of both aliphatic and aromatic fractions show a series of resolved peaks, and a characteristic "hump" termed the unresolved complex mixture or UCM. The UCM consists of

coeluting naphthenic (cycloalkanes), naphtho-aromatic and aromatic hydrocarbons in total petroleum, with both F_1 and F_2 fractions containing a UCM (Blumer *et al.*, 1970). The chromatograms of the aliphatic hydrocarbon fraction of oil characteristically show a homologous series of resolved normal alkane and isoprenoid (branched alkane) peaks with odd and even carbon chain length hydrocarbons in approximately equal amounts (Blumer, 1970; Blumer and Sass, 1972). These resolved peaks, projecting above a characteristic "hump" in the UCM, consist primarily of cycloalkanes (naphthenes) in the F_1 fraction (Fossato and Siviero, 1974; Farrington and Meyers, 1975; Teal and Farrington, 1977; NAS, 1980) (Fig. 11).

Fresh oil inputs, then, are signified by a chromatogram with a well resolved n-alkane and isoprenoid homologous series of peaks over a definite UCM. The fresher the oil, the wider the range of n-alkanes within the series from n- C_{10} or C_{12} and extending to very heavy molecular weight n-alkanes of n- C_{36} to n- C_{40} , and with a relatively greater proportion of resolved peaks to UCM (Fig. 12A). Since the low molecular weight n-alkanes (resolved peaks) are volatilized and degraded most rapidly (Walker *et al.*, 1975), moderately degraded oil is characterized by heavier molecular weight n-alkanes (n- C_{20} or n- C_{24} to n- C_{40}) over a UCM (Fig. 10). As the cycloalkanes comprising the UCM are degraded only very slowly (Farrington and Meyers, 1975; Teal and Farrington, 1977), a UCM with few resolved peaks is characteristic of highly degraded petroleum hydrocarbons from chronic petrogenic inputs (VanVleet and Quinn, 1977; Burns and Teal, 1979; Atlas *et al.*, 1981) (Figs. 10C and 12B). Due to a large UCM in moderately or heavily contaminated samples, the biogenic hydrocarbons may be overwhelmed by petrogenic hydrocarbons and, although present, are not measurable (Teal and Farrington, 1977).

Petrogenic hydrocarbons can, therefore, be distinguished from biogenic

hydrocarbons both by the presence or absence of a UCM in either or both of the F_1 and F_2 fractions, as well as by differences in the ratio of odd to even n-alkanes in the F_1 fraction. The carbon preference index (CPI) is a quantification of this odd to even carbon chain length ratio for n-alkanes between $n-C_{20}$ and $n-C_{32}$. Because petroleum hydrocarbons contain approximately equal amounts of even and odd n-alkanes, the CPI for petrogenic hydrocarbons is close to unity. CPI values from 1.0 to 2.0 are, therefore, indicative of samples containing predominately petrogenic hydrocarbons; so much so that the predominately odd chain length biogenic n-alkanes are overwhelmed. CPI values from 2.0 to 3.0 are indicative of samples moderately contaminated with oil, while CPI values from 3.0 to 4.0 indicate only slight oil contamination. CPI values greater than 4.0 indicate relatively uncontaminated samples containing predominately biogenic hydrocarbons.

The presence of the isoprenoids pristane and phytane is also often used as an indicator of oil contamination. Pristane is a common biogenic hydrocarbon derived from zooplankton (Blumer *et al.*, 1971), while both pristane and phytane in generally equal amounts are found in oil hydrocarbons. Thus, large concentrations of pristane with little phytane is indicative of relatively uncontaminated samples, while large amounts of both indicate oil contamination.

From the above considerations, the oysters from the St. Mary's River used as controls in this study are indeed largely uncontaminated even though there is a small UCM present in both the F_1 and F_2 fractions, indicating the presence of highly degraded petroleum (Fig. 7A and B). From inspection of the chromatogram of the F_1 fraction (aliphatics) of the whole body tissues, there is clearly a strong predominance of odd-carbon chain lengths (Fig. 7A). The typically algal biogenics $n-C_{15}$ and $n-C_{17}$ are present along with considerable pristane and virtually no phytane, and there is an extremely large $n-C_{31}$ peak

indicative of terrigenous inputs. A CPI of 7.1 (Table IV) further substantiates the biogenic nature of the hydrocarbons present.

The concentrations of total hydrocarbons and the CPI values for the oysters from the baseline study (Phase I) (Table IV) would indicate relatively low, yet ubiquitous, contamination of the mid-Chesapeake Bay by petroleum hydrocarbons. It should be noted, however, that all the values in Table IV are probably low by a factor of 4. This is due to the accepted practice of utilizing a reporting integrator to calculate the areas under the chromatogram peaks. Hand computation of the areas on a few selected chromatograms indicated that the integrator was systematically underestimating the actual area of the UCM's, particularly on chromatograms with a large UCM. Injection of known weights of crude oil, No. 2 fuel oil, and No. 6 fuel oil with subsequent quantification by the integrator confirmed that the integrator did indeed considerably underestimate the area of the UCM by an average factor of approximately 4. Although automatic integration is commonly used and widely accepted, recent work by Farrington et al. (1982b) substantiates that automatic integration is an unreliable method for quantifying chromatogram areas of petroleum hydrocarbons due to the large and variable UCM. Farrington and his group currently do not use automatic integration, but measure the UCM by planimetry (Farrington et al., 1982b; and Farrington, personal communication). We are currently investigating a quantification method using automatic integration to identify and quantify the resolved peaks only, while quantifying the UCM by planimetry. Therefore, for the present study, a more accurate estimate of the actual hydrocarbon concentrations can be obtained by applying a correction factor of 4 to the values. These corrected values are presented for comparison in Table IV. Comparison of these corrected values to concentrations in mussels and oysters from harbors, bays and urban coastal areas which receive wastewater discharges, and where shipping traffic is moderately

heavy, strongly suggest that indeed these correct concentrations are more likely correct. For instance, concentrations on the order of 100 to 300 $\mu\text{g/g}$ dry weight and higher have been frequently observed in Venice Lagoon (Fossato and Siviero, 1974; Fossato and Canzonier, 1976), Galveston ship channel and Galveston Bay (Erhardt, 1972), Boston Harbor (Farrington *et al.*, 1980; Farrington *et al.*, 1982a), and a number of harbors, bays and coastal areas of California (Risebrough *et al.*, 1980). Four chromatograms representative of the baseline study are presented in Figures 8-11. Examination of Fig. 8A from Bloody Point Bar suggests a low level of total and petrogenic hydrocarbons. The moderately low CPI of 2.0, coupled with the clearly distinguishable homologous series of n-aliphatics from C_{22} - C_{36} , is indicative of a moderate petrogenic hydrocarbon input. The relatively small UCM to resolved peak ratio indicates that these oysters were exposed to fresh oil and that a low chronic input is present at this site.

In contrasting this to the chromatogram of background monitors from Tolly Point, it can be seen (Fig. 9A) that a clearly defined homologous series is not as obvious and that there is a somewhat larger UCM. This lack of a homologous series along with a CPI of 3.5 indicates that these oysters were exposed to very low levels of fresh oil and only slightly higher levels of degraded oil from chronic inputs than were the Bloody Point monitoring organisms. Note the rather high concentrations of odd carbon numbered aliphatics (from C_{27} - C_{31}) indicative of a high input of terrigenous biogenics.

Figure 10, on the other hand, demonstrates moderately high levels of degraded hydrocarbons from chronic sources as well as relatively fresh petrogenic inputs. This is indicated in the over-all patterns of resolved n-alkane peaks (relatively fresh inputs) emerging from a large UCM (degraded

oil). The clear homologous series of n-alkanes coupled with a moderately low UCM indicate that the hydrocarbons present are petrogenic and not biogenic. In fact, the level of petrogenic hydrocarbons has obliterated the pattern of biogenics that could be seen in Figures 7 and 9. A comparison of this chromatogram with that of No. 6 fuel oil (Fig. 19) suggests that the principle contributor to these hydrocarbons is indeed No. 6 fuel oil that has lost the highly volatile fraction during the initial stages of degradation.

The chromatograms of oysters taken from the baseline study at the mouth of the Patapsco clearly indicate a high level of petrogenic contamination. Here we find a very large UCM indicating large chronic nonpoint inputs of degraded petroleum hydrocarbons. Clearly resolved peaks for the homologous series of n-alkanes indicate that fresh inputs of petrogenic hydrocarbons are also present. Comparison with Fig. 19 suggests that both heavy fuel oils and diesel oil may contribute substantially to the overall petrogenic hydrocarbon burden of this area. Note that this chromatogram is reduced by a factor of 2 in order to maintain the peaks on scale and would thus appear twice as large if presented at the same scale as Fig. 7-11. In spite of the high petrogenic hydrocarbon levels, there is a very high input of both algae and terrigenous biogenic hydrocarbons indicating high productivity (Erhardt and Heinemann, 1975), and suggesting a nutrient rich environment.

Analysis of the hydrocarbons extracted from the baseline biomonitors suggests that there is a substantial input of petrogenic hydrocarbons into the Bay from the Patapsco River and points further north along the western shore and from the Susquehanna River. The corrected concentration of these inputs is comparable to hydrocarbon levels reported for other industrial and shipping harbors. As expected, there is a dilution effect seen in

monitors placed further down the Bay.

It is not possible to state conclusively from the baseline data what proportion of the hydrocarbon found in the anchorage area originate from vessels at anchor. The elevated levels found at Brickhouse Bar, however, suggest that this eastern shore station is receiving input from the anchorage. Due to the Coriolis effect, the net outward flow from the upper Bay is displaced toward the western shore of the Bay as it passes through the anchorage. This outward flow of water along the western shore, plus the contributions of the Severn and South River, should result in higher petrogenic hydrocarbon levels on the western shore as seen, for example, at the Bloody Point and Cedarhurst Stations (Table 1, Fig. 4). The monitors at Brickhouse Bar, however, show both a lower CPI (1.6) and a higher total corrected hydrocarbon level (134 $\mu\text{g/g}$ dry weight) than the equivalent western shore station at Tolly Point (CPI = 3.5; Σ hydrocarbon = 118 $\mu\text{g/g}$ dry weight). This suggests to us that there is a measurable increment in petrogenic hydrocarbon input due to vessels at anchor or in transit through the Annapolis Roads. In the case of the Brickhouse Bar station, this increment (18 $\mu\text{g/g}$ dry weight) is approximately 15% of the total hydrocarbon concentration and is clearly petrogenic in nature (CPI = 1.6).

Phase II. Short-Term 48 Hour Biomonitoring:

Examination of Figures 12-19 indicates that the ctenidia of oysters do indeed come to rapid equilibrium with hydrocarbons in the water column resulting in a substantial magnification of water concentrations. Notice the relatively high levels accumulated and the clearly discernable n-alkane homologous series in the F_1 fraction. Note also that the hydrocarbon concentration in the F_2 fraction is considerably lower than that found in whole oyster bodies. We speculate that the more soluble nature of the

F₂ fraction results in a rapid dispersion and homogenous mixing throughout the water column, reducing the concentration of this fraction in surface waters.

Figure 12 illustrates three typical chromatograms of background levels of aliphatic hydrocarbons extracted from oyster gills exposed to the waters of Annapolis Roads. A typical low background concentration is exemplified by Figure 12A, the second retrieval from Brickhouse Bar (8/13/81). Notice the relatively small contribution of the UCM to the overall area and the small, but clearly evident, homologous series of n-alkanes that typify recent petrogenic input. In contrast, Figure 12B demonstrates an elevated background hydrocarbon level present at Cedarhurst on 8/11/81. Note the high level of biogenics (odd carbon numbers) and the clearly distinguishable homologous series indicative of petrogenic hydrocarbon input. Figure 12C (third retrieval 8/15/81 from Brickhouse Bar) illustrates the third major pattern in background levels. The total hydrocarbon level is somewhat higher than during the first retrieval (3.69 vs 3.06 µg/g dry weight) but the lack of a defined homologous series and larger UCM demonstrates that it is a mixture of highly degraded hydrocarbons from chronic inputs.

With the background levels depicted in Figure 12 as a reference, the chromatograms depicted in Figure 13 clearly depict a spill or discharge incident. Chromatograms of gill monitors from this station (Thomas Point Shoal) collected on 8/11/81 and 8/13/81 look very much like Figure 12A. In contrast, the gill monitors collected at the next sampling period (8/15/81) demonstrate a very recent input of No. 2 and No. 6 fuel oil, possibly from one incident. The large clearly defined homologous series, along with the presence of low carbon number alkanes indicates little volatilization or degradation of the oils had occurred. The gill monitors collected at this same station 48 hours later continue to show fresh inputs of petrogenic

hydrocarbons (Fig. 13B). Notice, however, that now the peaks have shifted to higher carbon number and are more suggestive of degraded No. 6 fuel oils (Fig. 19).

A spill event or discharge on or about 8/17/81 impacting on the monitoring station at Brickhouse Bar is depicted in Figure 14A. The nearly immediate impingement of the oil on these organisms is indicated by the homologous series extending down to low carbon numbered alkanes, and by the extraordinarily high ratio of resolved peak height to UMC height. Examination of chromatograms of gill monitors collected earlier at this station (Figs. 12A and C) further document that this was a recent event and not the result of a high overall background at this station.

In contrast, the chromatogram depicted in Figure 19B indicates a spill event in which highly degraded oil, with only a small contribution of fresh #2 fuel oil, impacted on the monitoring station collected on 8/15/81 at Bloody Point Bar. Notice especially the very large UCM with relatively insignificant peaks in the homologous series, suggesting very highly weathered oil. As the monitors collected prior to 8/15/81 at this station show only low to moderate background levels of petrogenic hydrocarbons, we suggest that this oil was not in the environment prior to 8/13/81 but rather that it was introduced in a bilge pumping event. Further evidence in support of this supposition can be found in the very heavy, late-eluting hydrocarbons that would be characteristic of the heavy lubricating oils that might also find their way into bilge waters.

Minor spill events can be seen in the chromatograms presented in Figure 15. The gill monitors collected on 8/11/81 from Cedarhurst (Fig. 15A) show moderately high levels of petrogenic hydrocarbon, suggesting a mixture of degraded No. 2 fuel oil and fresher No. 6 fuel oil. As evidenced by the large UCM and high resolved peaks in the homologous series, this oil is more

by the gill monitors retrieved from Hacket Point on 8/13/81 (Fig. 15B). This chromatogram (Fig. 15B) is characteristic of a more recent input as demonstrated by larger resolved peaks in relation to the UCM and the presence of lower carbon number alkanes.

The use of oysters and other bivalves to monitor petroleum hydrocarbons in aquatic environments is now firmly established (Goldberg *et al.*, 1978; Philips, 1980; NAS, 1980; Burns and Smith, 1981; Farrington *et al.*, 1982a). To date bivalves have been used only as long-term monitors since it takes several weeks or more for the various body tissues of transplanted biomonitors to equilibrate with the concentrations of hydrocarbons in the water column (Stegeman and Teal, 1973; Young *et al.*, 1976; Burns and Smith, 1981).

Uptake of both aliphatic and aromatic hydrocarbons from ambient water by gill tissues, however, begins within minutes of exposure to water containing hydrocarbons (Lee *et al.*, 1972). Lee *et al.* (1972) hypothesize that hydrocarbon absorption takes place in the gill tissue micellar layer, and then only over time are these absorbed hydrocarbons passed on to other tissues via exchange equilibria (Hamelink *et al.*, 1971). Whole body tissues, therefore, integrate the fluctuating concentrations of hydrocarbons first absorbed by gill tissues. Thus, the analysis of whole body tissues may provide a record of the average ambient water hydrocarbon concentrations (Burns and Smith, 1981), while analysis of gill tissues may provide a record of the day to day fluctuations in these ambient concentrations.

Comparison of chromatograms of hydrocarbons in whole oyster tissues (Fig. 16A) with those in gill tissues collected at the same station from four successive retrievals (Figs. 16B-E) does indeed confirm that whole body tissues integrate over time, while gill tissues display the day to

day fluctuations in both the quantity and quality of water borne hydrocarbons. Figs. 16A and B are chromatograms of the hydrocarbons in whole oyster tissue and gill tissues, respectively, from the same group of oyster monitors. The body tissues (Fig. 16A) clearly indicate a much higher mean hydrocarbon concentration from fresher petrogenic inputs than the gill tissues collected simultaneously or on the next two successive retrievals (Figs. 16B-D). All show low level, relatively degraded petroleum hydrocarbons and algal biogenics. The chromatogram of the hydrocarbons in the gill monitors from the fourth retrieval at this same station (Fig. 16E), however, shows a rather large recent input of fresh fuel oils. Although only one spill event occurred during the eight days monitored at this station (Figs. 16B-E), spills or discharges must occur with some regularity for the whole body tissues to show such elevated concentrations (Fig. 16A) (Stegeman and Teal, 1973; Fossato and Canzonier, 1976).

In contrast, the large pulse of petroleum hydrocarbons evidenced by the chromatograms of gill monitors from Bloody Point Bar (Fig. 14B), presumably from a bilge pumping incident, is not reflected in the chromatograms of hydrocarbons in the whole body tissues from monitors at this station (Fig. 17A) which exhibit a relatively low mean petroleum hydrocarbon concentration. The pulsed nature of this input is clearly evident as the gill monitors from retrievals both preceding and following this date show only relatively low concentrations of petroleum hydrocarbons. Pulsed inputs of this nature must have been relatively infrequent during the three weeks monitored by the whole body tissues in order for these tissues to demonstrate the low concentrations seen in Fig. 17A.

Comparison of the chromatograms of the hydrocarbons in whole body monitors and gill monitors also show consistent qualitative differences. Biogenic hydrocarbons ($n\text{-C}_{15}$, $n\text{-C}_{17}$, pristane) appear generally absent from whole body tissues (Fig. 9A, 16A, & 17A) but are clearly present in

gill tissues (Fig. 12, 16B, & 17B). The presence of these biogenics in the whole body tissues of St. Mary's River control oysters (Fig. 7A), however, suggests that these may be present in the whole body monitors, but obscured by the larger UCM present in the samples. The very large biogenic $n\text{-C}_{31}$ peak appearing in most of the whole body samples (Figs. 9A, 16A & 17A), and its absence from gill samples (Figs. 12, 13, & 17B), may result from either its selective uptake, or its selective retention as the control oysters also possess this peak (Fig. 7A).

Even though biogenics are clearly evident in the gill monitors, the relatively higher concentration on a dry weight basis, as well as more clearly resolved peaks due to a lower UCM, facilitates identification of the petroleum hydrocarbons present.

It is clear that the short-term biomonitor is an effective method of monitoring day to day changes in hydrocarbon concentrations. The gill tissues respond rapidly to spill or discharge incidents and provide a clear picture of the hydrocarbons present in the water. The gills also depurate or transfer the accumulated hydrocarbons into the body over short time periods and are thus extremely useful in answering both qualitative and quantitative questions about hydrocarbon concentrations that might fluctuate widely in time, i.e. small repetitive discharges from vessels at anchor or terrigenous inputs following heavy rainfall.

We feel that a combination of long-term (2-4 weeks) and short-term (24-48 hours) monitors can provide information on the overall integrated hydrocarbon levels as well as daily fluctuations indicating specific short-term input.

Sediments:

Generally the sediment hydrocarbon concentrations reflected the hydrocarbon concentrations of the water column above them. Thus, the Patapsco River station sediments had the highest concentrations (Fig. 18A) and the southern eastern shore stations the lowest (Fig. 18B) with concentrations decreasing down the Bay. All sediment samples had considerable amounts of the $n-C_{23}$ - $n-C_{31}$ terrigenous biogenics and a UCM which is indicative of highly degraded petroleum hydrocarbons (Wehmiller and Lethen, 1975; Teal and Farrington, 1977). The major difference between the hydrocarbons in the sediments from the different stations was the size of the UCM (Fig. 18), and hence, the amount of degraded petroleum present. The Bloody Point Bar and Brickhouse Bar stations had relatively "clean" sediments with a low UCM (Fig. 18B), while sediments from the other stations had a UCM similar to that observed at the Patapsco River station (18A). The relative absence of petroleum hydrocarbons in the sediments of the southern eastern shore stations (Fig. 18B), but presence in the corresponding western shore stations, probably results from circulation patterns in this part of the Bay. The western shore stations receive most of their sediments and hydrocarbons from fresher waters flowing down this shore, while the southern eastern shore stations receive much of their sediment from further down the Bay.

Biological Relevance:

Anderson (1977) reported that there is little agreement between tissue hydrocarbon levels and sublethal effects, therefore water levels are more appropriate in predicting these effects. In order to determine the significance of the hydrocarbon levels determined in oyster tissues, therefore, it is necessary to back calculate to the hydrocarbon concentrations found in the Bay waters. Burns and Smith (1981) have demonstrated that within the

range from 1 $\mu\text{g}/\ell$ to 400 $\mu\text{g}/\ell$ there is a linear relationship between lipid concentration and water concentration. The simplest model assumes bio-concentration is independent of other constituents in seawater and defines a bioconcentration factor K_{Bcf} as the concentration of hydrocarbons in animal tissue divided by concentration in surrounding water. Using the K_{Bcf} determined for oyster lipid by Stegman and Teal (1973), the hydrocarbon concentrations of the water were calculated at the Bay baseline monitoring stations. Values in the upper Bay ranged from a high value of 9.2 and 4.3 $\mu\text{g}/\ell$ at the Patapsco and Swan Point stations, respectively, to low values of 3.1 and 3.8 $\mu\text{g}/\ell$ at the Cedarhurst and Bloody Point Bar stations. Within the anchorage, both Tolly Point (4.7 $\mu\text{g}/\ell$) and Brickhouse Bar (5.59 $\mu\text{g}/\ell$) showed elevated values.

These values are comparable to those reported in the literature for coastal waters in urban industrial areas (Farrington *et al.*, 1980) and fall within the range of values (0.1 $\mu\text{g}/\ell$ to 22 $\mu\text{g}/\ell$) reported in the vicinity of oil loading docks (Burns and Smith, 1978).

Interpretation of the significance of these levels of hydrocarbons is difficult due to the lack of information on the effects of chronic low doses of oil (Connell and Miller, 1981). Some generalization, however, can be made. The impact of oil on marine and estuarine ecosystems is governed by several factors - physical, chemical and biological - in addition to the inherent complexity of crude and refined petroleum. Many estuarine organisms are living near the limit of their tolerance range and any additional stress imposed by even low levels of petroleum could eliminate sensitive organisms from the estuary (Odum, 1970). In variable environments like the Chesapeake, responses to stresses such as oil are also variable (Evans and Rice, 1974). The ability of estuarine communities to endure oil stress will be influenced by the variability of natural stress and by differences in vulnerability of different stages in an organism's life cycle, its state of nutrition, and other factors.

There is an enormous body of literature on the effects of high level oil contamination on organisms (see reviews by Moore and Dwyer, 1974; Anderson et al., 1974; Hyland and Schneider, 1976; Johnson, 1977; Wolfe, 1977; Connell and Miller, 1981). Data collected after major spill incidents provide us with a reasonably clear picture of the effects of large acute inputs (see reviews by Sanders et al., 1980; Kineman et al., 1980). Laboratory studies have increased our understanding of sublethal effects, but considerably more information on the interaction of sublethal oil concentrations and other environmental stresses is required (Widdows et al., 1982). The vast majority of studies reporting effects on estuarine organisms is for oil concentrations greater than 100 $\mu\text{g}/\text{l}$ - the upper Chesapeake contains only one-tenth that level.

Widdows et al. (1982), however, presents extensive data on the effects of low petroleum hydrocarbon concentrations on mussels. At sea water concentrations of 30 μg crude oil/ l seawater, they noted significant reduction in feeding rates and elevated respiratory rates. Mussels exposed to only 30 $\mu\text{g}/\text{l}$ also showed marked reductions in energy available for growth (scope for growth), and structural and functional changes in their digestive system (Widdows et al., 1982). In addition, Widdows et al. (1982) demonstrated substantial reduction in the scope for growth at tissue hydrocarbon concentrations comparable with those recorded in this study. Mussels with aromatic hydrocarbon concentrations equal to those from oyster monitors at Bodkin Neck or Swan Point, for example, showed a 40% reduction in their scope for growth.

From the data reported herein, we conclude that the petroleum hydrocarbon concentrations in the upper Chesapeake Bay do indeed exceed concentrations observed in "clean" environments; however, these concentrations fall well within the range of concentrations reported in other heavily utilized

waterways receiving wastewater discharges. These hydrocarbons in the upper Chesapeake Bay are dispersed down the estuary providing elevated background throughout the Annapolis Roads area. Nevertheless, vessels at anchor within the Roads appear to contribute measurable quantities of petroleum hydrocarbons. On the basis of the data from the Brickhouse Bar station, we estimate this contribution to be approximately 15% of the total hydrocarbon load of the waters in the anchorage.

The concentrations of petroleum hydrocarbons observed within the anchorage area are currently well below those that would result in acute biological effects, but recent evidence suggests that these concentrations are in excess of levels that can produce sublethal effects in bivalve molluscs. In this light, even slightly elevated background concentrations of pollutants may significantly affect natural populations.

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Table I. Location of Sampling Stations For Bay Base Line Biomonitoring and 48-hour Short Term Monitors

| Station Number | Location | |
|----------------|---------------------------------------|--------------------|
| | Geographic Reference | Map Coordinates |
| I 1 | Bodkin Neck Patapsco River | 76°26'W 39°10'N |
| I 2 | Swan Point Eastern Shore | 76°17'W 39°8'N |
| I 3, II 3 | Hackett Point Bay Bridge West | 76°24'W 38°59'N |
| I 4, II 4 | Stevensville Bay Bridge East | 76°22'W 38°58'N |
| I 5, II 5 | Tolley Point Mouth of Severn River | 76°26'W 38°56'N |
| I 6, II 6 | Brickhouse Bar Kent Island | 76°23'W 38°55'N |
| I 7 | Chinks Point Severn River | 76°29'W 38°58'N |
| I 8, II 8 | Thomas Point Shoal South River | 76°26'W 38°54'N |
| I 9, II 9 | Cedarhurst West River | 76°28'W 38°50'N |
| I 10, II 10 | Bloody Point Bar South Kent Island | 76°24'W 38°50'N |

Table II. Station Recovery of Phase I baseline stations (I,#) and the three sets of Phase II oyster gill monitor stations (II,#)

| Station | Date Set | Date Recovered |
|---------------|---------------------------------|----------------------------------|
| I 1 | 7/23/81 12 oysters each station | 8/11/81 All recovered |
| I 2 | 7/23/81 12 oysters each station | 8/11/81 All recovered alive |
| I 3, II 3-1 | 7/23/81 12 oysters each station | 8/11/81 Station not recovered |
| I 4, II 4-1 | 7/23/81 12 oysters each station | 8/11/81 Station not recovered |
| I 5, II 5-1 | 7/23/81 12 oysters each station | 8/11/81 Crash damage/1 recovered |
| I 6, II 6-1 | 7/23/81 12 oysters each station | 8/11/81 All recovered |
| I 7, | 7/23/81 12 oysters each station | 8/11/81 All recovered |
| I 8, II 8-1 | 7/23/81 12 oysters each station | 8/11/81 All recovered |
| I 9, II 9-1 | 7/23/81 12 oysters each station | 8/11/81 All recovered |
| I 10, II 10-1 | 7/23/81 12 oysters each station | 8/11/81 All recovered |
| II 3-2 | 8/11/81 12 oysters each station | 8/13/81 Station not recovered |
| II 4-2 | 8/11/81 12 oysters each station | 8/13/81 All recovered |
| II 5-2 | 8/11/81 12 oysters each station | 8/13/81 All recovered |
| II 6-2 | 8/11/81 12 oysters each station | 8/13/81 All recovered |
| II 8-2 | 8/11/81 12 oysters each station | 8/13/81 All recovered |
| II 9-2 | 8/11/81 12 oysters each station | 8/13/81 All recovered |
| II 10-2 | 8/11/81 12 oysters each station | 8/13/81 All recovered |
| II 3-3 | 8/13/81 12 oysters each station | 8/15/81 All recovered |
| II 4-3 | 8/13/81 12 oysters each station | 8/15/81 Station not recovered |
| II 5-3 | 8/13/81 12 oysters each station | 8/15/81 All recovered |
| II 6-3 | 8/13/81 12 oysters each station | 8/15/81 All recovered |
| II 8-3 | 8/13/81 12 oysters each station | 8/15/81 All recovered |
| II 9-3 | 8/13/81 12 oysters each station | 8/15/81 All recovered |
| II 10-3 | 8/13/81 12 oysters each station | 8/15/81 All recovered |
| II 3-4 | 8/15/81 12 oysters each station | 8/17/81 Station not recovered |
| II 4-4 | 8/15/81 12 oysters each station | 8/17/81 Station not recovered |
| II 5-4 | 8/15/81 12 oysters each station | 8/17/81 All recovered |
| II 6-4 | 8/15/81 12 oysters each station | 8/17/81 All recovered |
| II 8-4 | 8/15/81 12 oysters each station | 8/17/81 All recovered |
| II 9-4 | 8/15/81 12 oysters each station | 8/17/81 All recovered |
| II 10-4 | 8/15/81 12 oysters each station | 8/17/81 All recovered |

Table IIIA. Percent recovery of F_1 aliphatics from spiked samples of St. Mary's River oysters.

| Compound | ug Added | ug Recovered | ug Recovered | \bar{X} % Recovered |
|------------|----------|---------------------|---------------------|--------------------------|
| | | -ug in Control A | -ug in Control B | |
| C12 | 0.9696 | 0.340 | 0.397 | 38.0 |
| C14 | 0.9916 | 0.686 | 0.713 | 70.5 |
| C15 | 1,8560 | 1.797 | 1.832 | 97.7 |
| C16 | 1.0053 | 0.768 | 0.806 | 78.3 |
| C18 | 0.9155 | 0.800 | 0.823 | 88.6 |
| C20 | 3.0000 | 3.011 | 3.100 | 101.8 |
| C22 | 1.0213 | 0.961 | 0.978 | 99.9 |
| C24 | 0.9865 | 0.871 | 0.879 | 88.9 |
| C26 | 1.0000 | 0.878 | 0.882 | 88.0 |
| C28 | 1.0000 | 0.802 | 0.827 | 81.4 |
| C30 | 1.0000 | 0.801 | 0.839 | 82.0 |
| C32 | 1.0000 | 0.832 | 0.838 | 83.5 |
| C34 | 1.0000 | 0.866 | 0.904 | 88.5 |
| C36 | 1.0780 | 0.728 | 0.761 | 68.9 |
| Androstane | 1.7490 | 1.074 | 1.100 | 62.1 |

Mean overall 80.88%

SD 16.04%

Table IIIB. Percent recovery of F_2 (aromatics) from spiked samples of St. Mary's River oysters.

| Compound | ug Added | ug Recovered | ug Recovered | \bar{X} % Recovered |
|-------------------|----------|---------------------|---------------------|--------------------------|
| | | -ug in Control A | -ug in Control B | |
| Hexamethylbenzene | 169.0 | 46 | 66 | 33.1 |
| Hexaethylbenzene | 157.2 | 112 | 104 | 68.0 |
| Anthracene | 170.0 | 75 | 70 | 43.3 |
| n C22 | 169.3 | 0.00 | 0.00 | 0.00 |
| n C15 | 158.7 | 0.00 | 0.00 | 0.00 |
| Androstane | 146.0 | 0.00 | 0.00 | 0.00 |

Mean overall 48.0%

SD 17.28%

Table IV. Hydrocarbons in oyster biomonitors from the Phase I baseline study (July-August 1982).

| Station | CPI | F ₁ levels | | F ₂ levels | | F ₁ + F ₂ | | Σ Hydrocarbons ug/g dry weight | Σ Hydrocarbons ug/g dry weight corrected |
|--------------------------------------------|-----|-----------------------|---------------|-----------------------|----------------|---------------------------------|----------------|-----------------------------------|------------------------------------------------|
| | | ug/g wet | ug/g dry | ug/g wet | ug/g dry | ug/g wet | ug/g dry | | |
| I1 - Bodkin Neck 76° 26'W: 39° 10'N | 1.6 | 5.5 | 49.6 | 619.1 | 1.7 | 10.7 | 116.9 | 60.3 | 241.2 |
| I2 - Swan Point 76° 17'W: 39° 8'N | 1.3 | 3.2 | 20.0 | 219.2 | 1.9 | 12.0 | 132.0 | 32.0 | 128.0 |
| I3 - Hackett Point 76° 24'W: 38° 59'N | - | - | - | - | - | - | - | - | - |
| I4 - Stevensville 76° 22'W: 38° 58'N | - | - | - | - | - | - | - | - | - |
| I5 - Tolley Point 76° 26'W: 38° 56'N | 3.5 | 3.7 | 21.2 | 272.9 | 1.5 | 8.6 | 108.9 | 29.7 | 118.8 |
| I6 - Brick House Bar 76° 23'W: 38° 55'N | 1.6 | 3.4 | 20.7 | 243.5 | 2.1 | 12.8 | 150.7 | 33.5 | 134.0 |
| I7 - Chinks Point 76° 29'W: 38° 58'N | 1.7 | 1.6 | 8.9 | 110.9 | 1.0 | 19.4 | 204.0 | 28.3 | 113.2 |
| I8 - Thomas Point 76° 26'W: 38° 54'N | 2.5 | 0.9 | 6.4 | 84.5 | 1.5 | 11.0 | 141.3 | 17.5 | 70.0 |
| I9 - Cedarhurst 76° 28'W: 38° 50'N | 1.9 | 2.6 | 18.3 | 242.4 | 1.9 | 14.5 | 127.2 | 32.8 | 131.2 |
| I10 - Bloody Point 76° 24'W: 38° 50'N | 2.0 | 1.5 | 10.1 | 103.6 | 2.1 | 14.0 | 144.4 | 24.1 | 96.4 |
| St. Mary's River Control Site | | | | | | | | | |
| Replicate 1 | 7.1 | 1.31 | 9.3 | 172.7 | 0.74 | 5.31 | 98.5 | 14.8 | 59.2 |
| Replicate 2 | 7.1 | 1.31 | 9.6 | 178.2 | 0.74 | 5.21 | 98.2 | 14.6 | 58.6 |
| $\bar{x} \pm$ S.D. | | 1.32± 0.03 | 9.47± 0.21 | 175.41± 3.88 | 0.74± 0.001 | 5.26± 0.10 | 98.34± 0.18 | | |

Table V. Hydrocarbons in the gill monitors (short-term, 48 hour, August 1982).

| Station | First Pickup August 11 µg/g tissue | | | | Second Pickup August 13 µg/g tissue | | | | Third Pickup August 15 µg/g tissue | | | | Fourth Pickup August 17 µg/g tissue | | | |
|-------------------------|------------------------------------------|------|------|----------------|-------------------------------------------|------|------|----------------|------------------------------------------|------|------|----------------|-------------------------------------------|------|------|----------------|
| | F ₁ | Wet | Dry | F ₂ | F ₁ | Wet | Dry | F ₂ | F ₁ | Wet | Dry | F ₂ | F ₁ | Wet | Dry | F ₂ |
| 3 Hacket Point | NR | NR | NR | NR | 3.11 | 41.3 | 1.08 | 14.0 | NR | NR | NR | NR | NR | NR | NR | NR |
| 4 Stevensville | 0.97 | 6.24 | 0.92 | 15.7 | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR |
| 5 Tolly Point | NR | NR | NR | NR | 1.51 | 20.4 | 0.90 | 12.1 | 0.46 | 6.18 | 1.46 | 18.0 | 1.38 | 12.6 | 0.64 | 7.35 |
| 6 Brickhouse Bar | 0.25 | 2.64 | 3.42 | 36.7 | 0.24 | 3.69 | 1.35 | 19.3 | 0.24 | 3.06 | 1.78 | 22.2 | 9.79 | 108 | 1.88 | 20.1 |
| 8 Thomas Point Shoal | 0.19 | 2.20 | 0.50 | 4.86 | 0.44 | 6.33 | 1.73 | 24.9 | 2.31 | 26.5 | 1.43 | 16.5 | 2.60 | 26.8 | 1.09 | 11.2 |
| 9 Cedarhurst | 0.43 | 5.13 | 1.94 | 7.36 | 0.20 | 5.36 | 1.77 | 20.8 | 0.93 | 11.0 | 1.60 | 18.8 | 1.85 | 24.8 | 1.90 | 25.4 |
| 10 Bloody Point Bar | 0.29 | 3.31 | 0.73 | 8.69 | 1.13 | 17.7 | 1.18 | 18.5 | 5.78 | 85.6 | 0.99 | 12.8 | 1.55 | 18.6 | 2.54 | 30.3 |

NR = Not Recovered

**FIGURE 1. AVERAGE LENGTH OF STAY OF COLLIERIES
IN ANNAPOLIS ANCHORAGE FROM JANUARY, 1979 THROUGH NOVEMBER, 1981.**

(From Ostrum and Anthony, 1982)

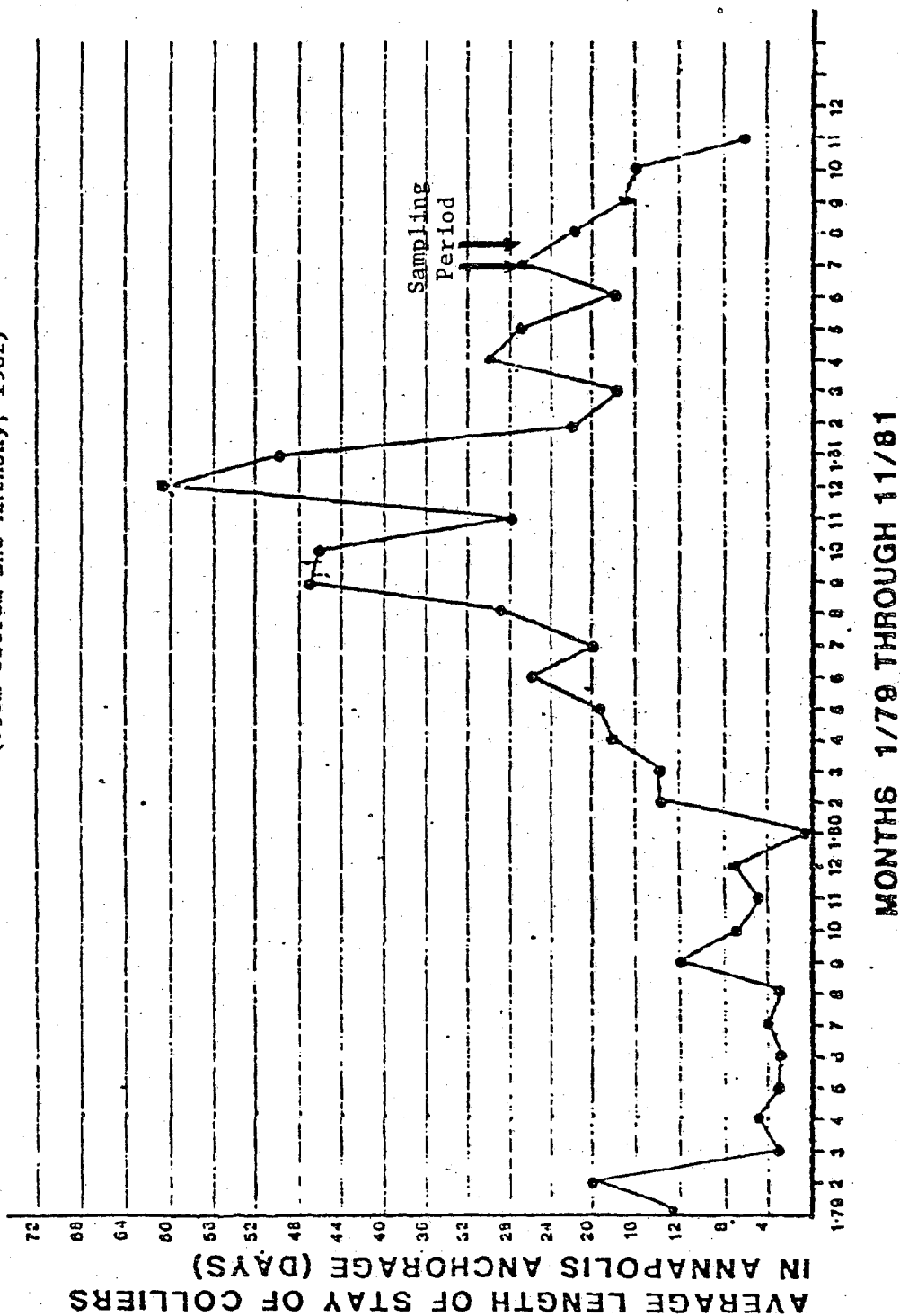
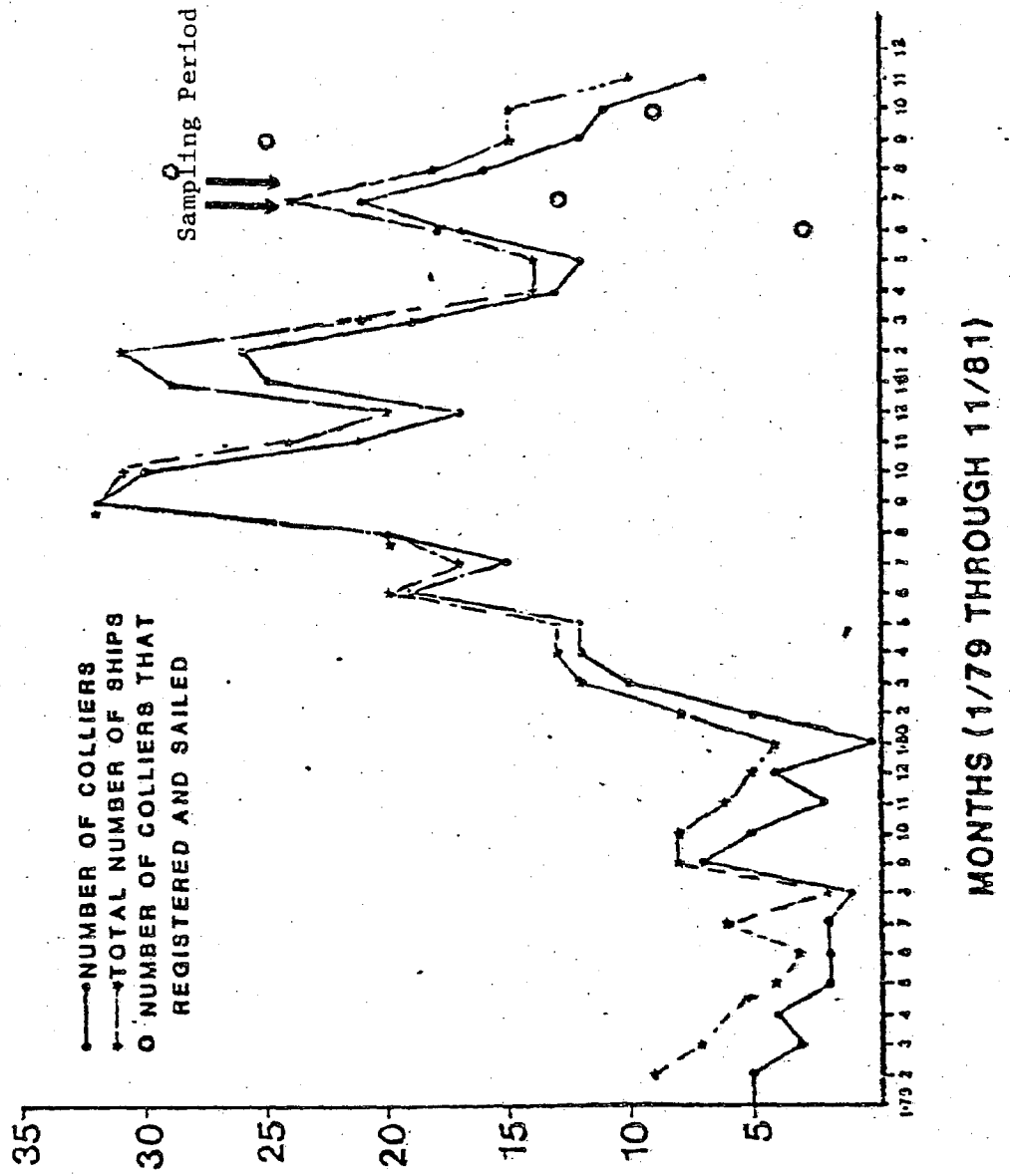


FIGURE 2 ESTIMATED TOTAL NUMBER OF SHIPS AND COLLIERIES
IN ANNAPOLIS ANCHORAGE FROM JAN, 1979 THROUGH NOV, 1981 .
NUMBER OF COLLIERIES THAT REGISTERED AND SAILED .

(From Ostrum and Anthony, 1982)



AVERAGE NUMBER OF SHIPS IN ANNAPOLIS ANCHORAGE
ON SIX RANDOMLY SELECTED DAYS FROM EACH MONTH

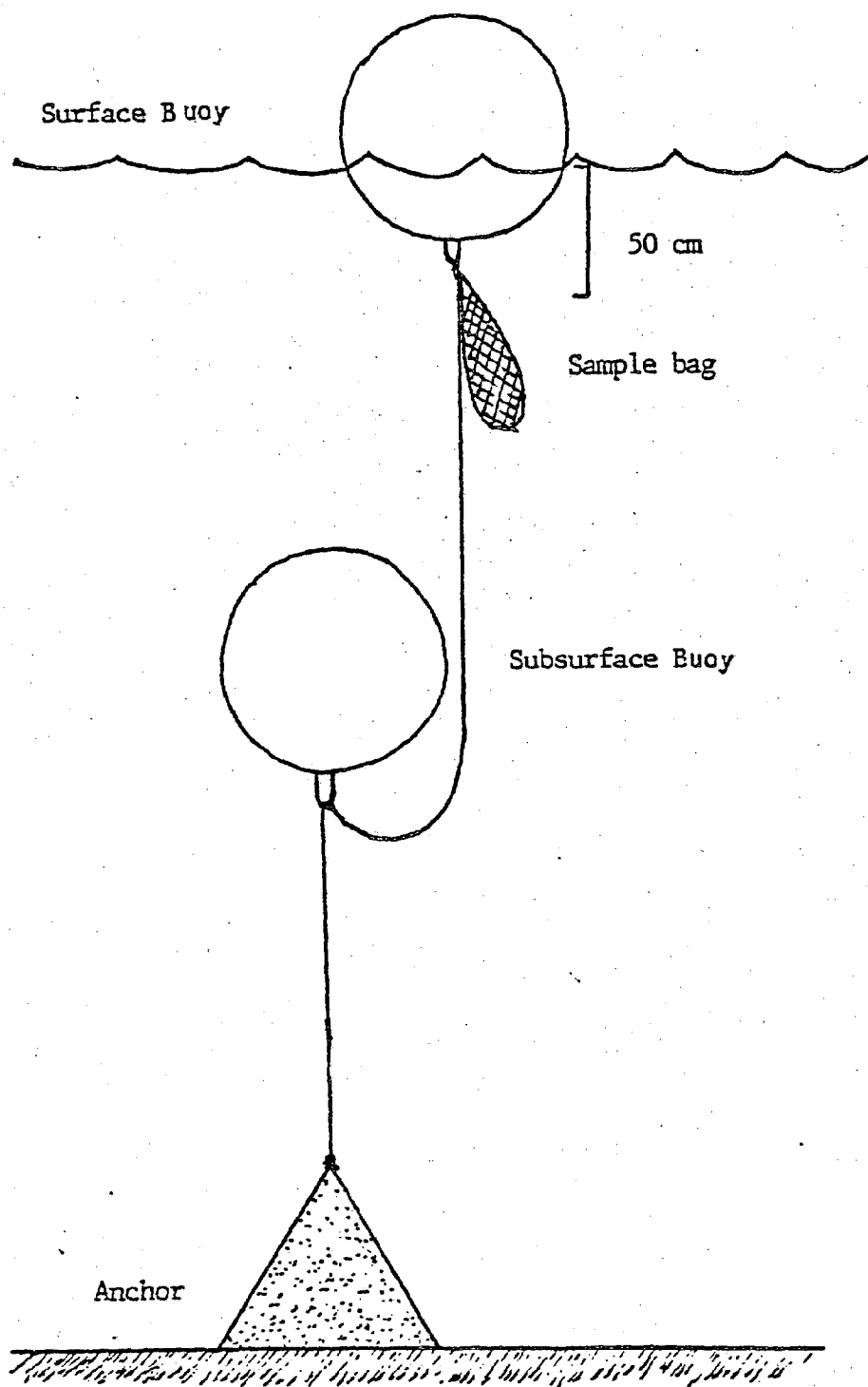


Fig. 3. Taut-line buoy system employed for incubating samples at each sampling station (after Young et al., 1976).

Figure 4. Middle region of the Chesapeake Bay showing the location of the anchorage for vessels awaiting entrance to the port of Baltimore. Biomonitoring stations 1-10 are indicated by circled numerals.

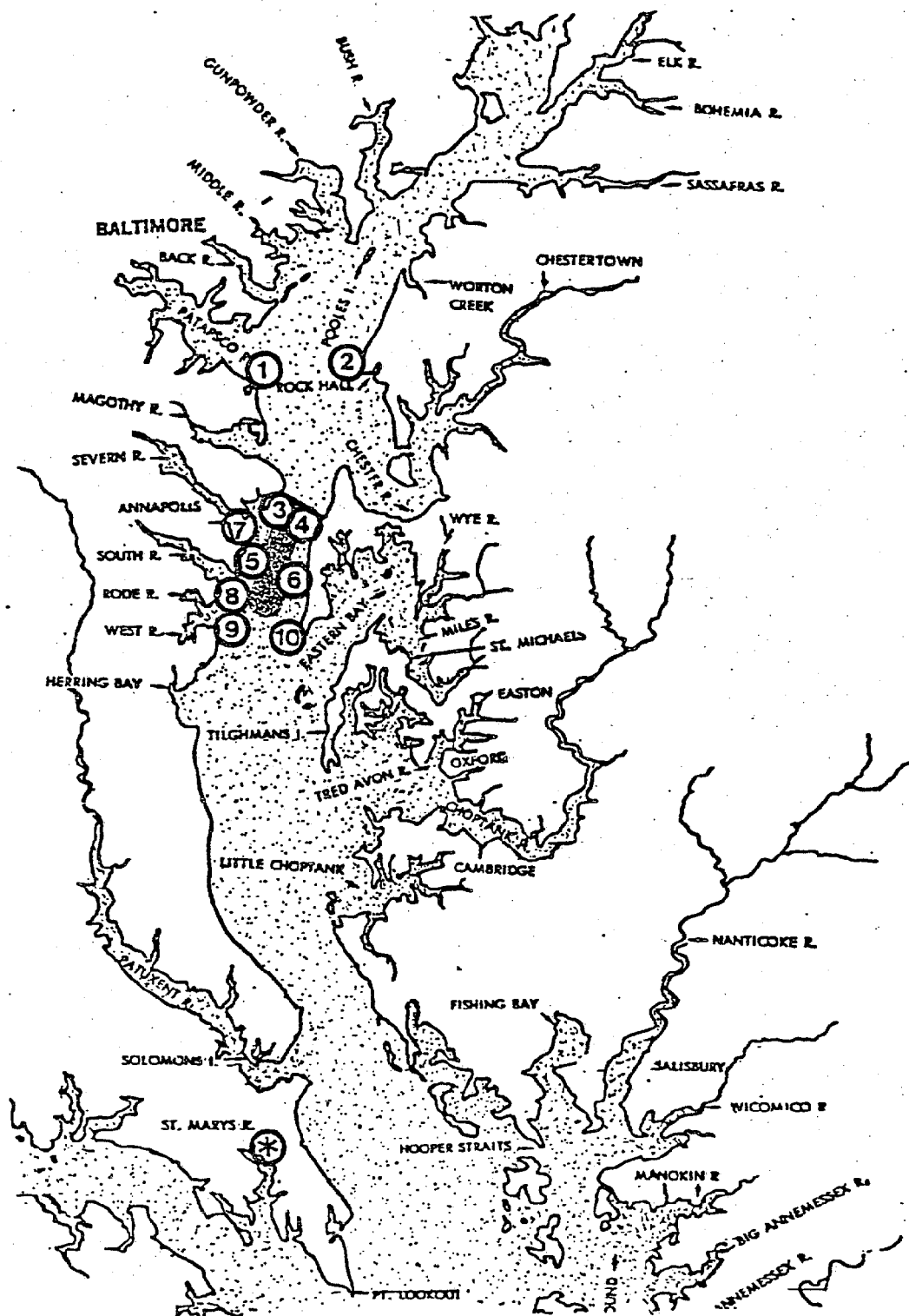


Figure 5. A. Chromatogram of the six component standard. B. Chromatogram of F_1 fraction containing the aliphatic (alkane-cycloalkane) hydrocarbons. C. Chromatogram of F_2 fraction containing the aromatic hydrocarbons. D. Chromatogram of F_3 fraction containing n-alkan-2-ones and methyl esters of n-fatty acids and steradienes.

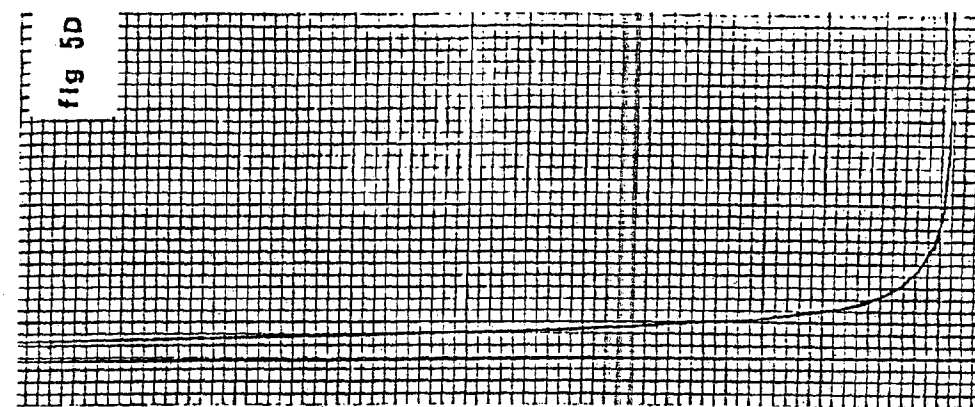
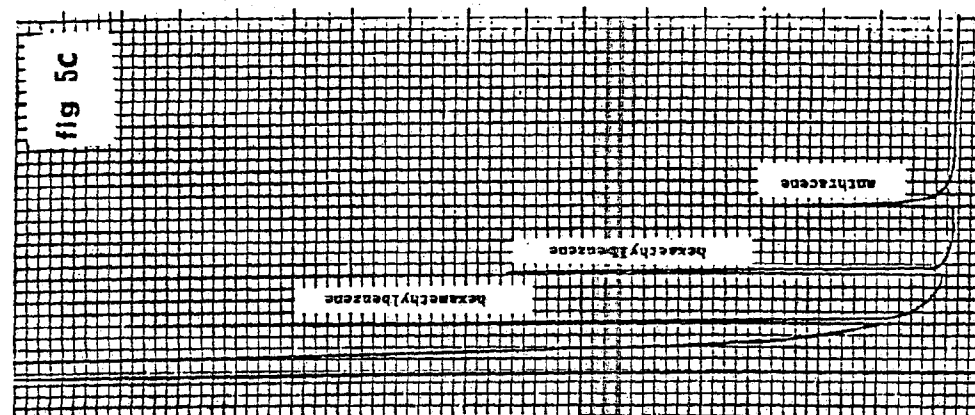
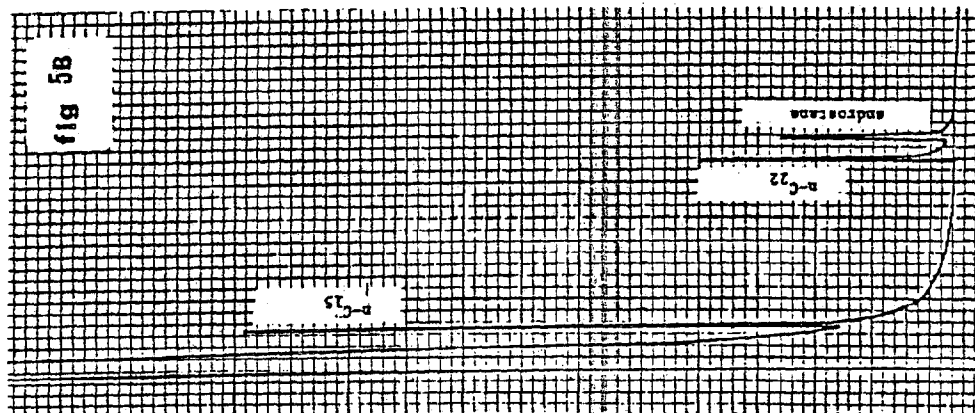
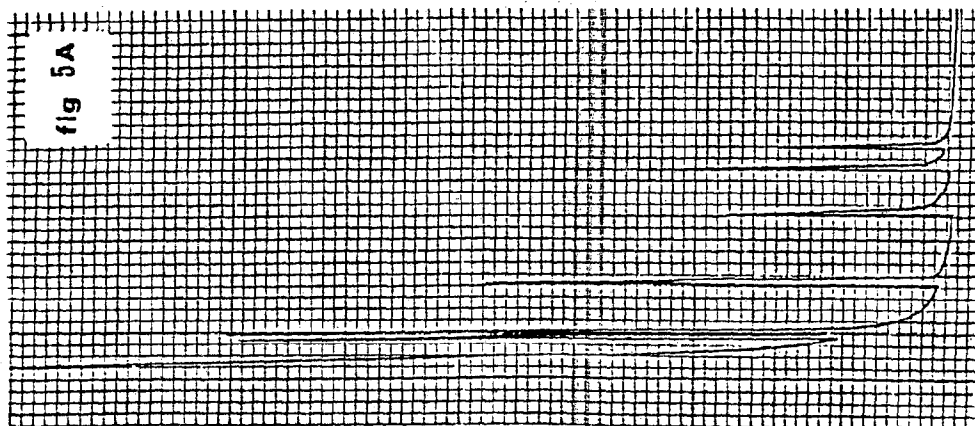


Figure 6. A. Representative chromatogram of a solvent blank.
B. Representative chromatogram of a procedural blank.
Contam. is an unidentified contaminant that coelutes with n-C₂₈.

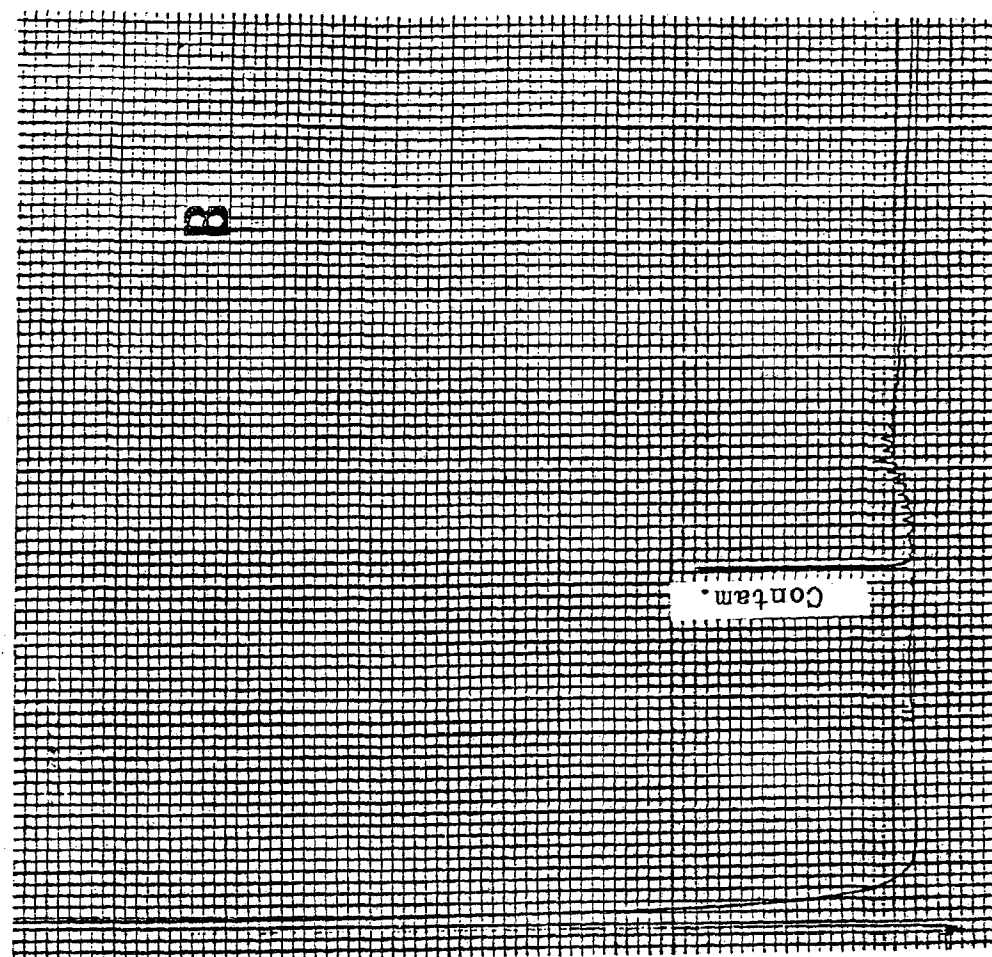
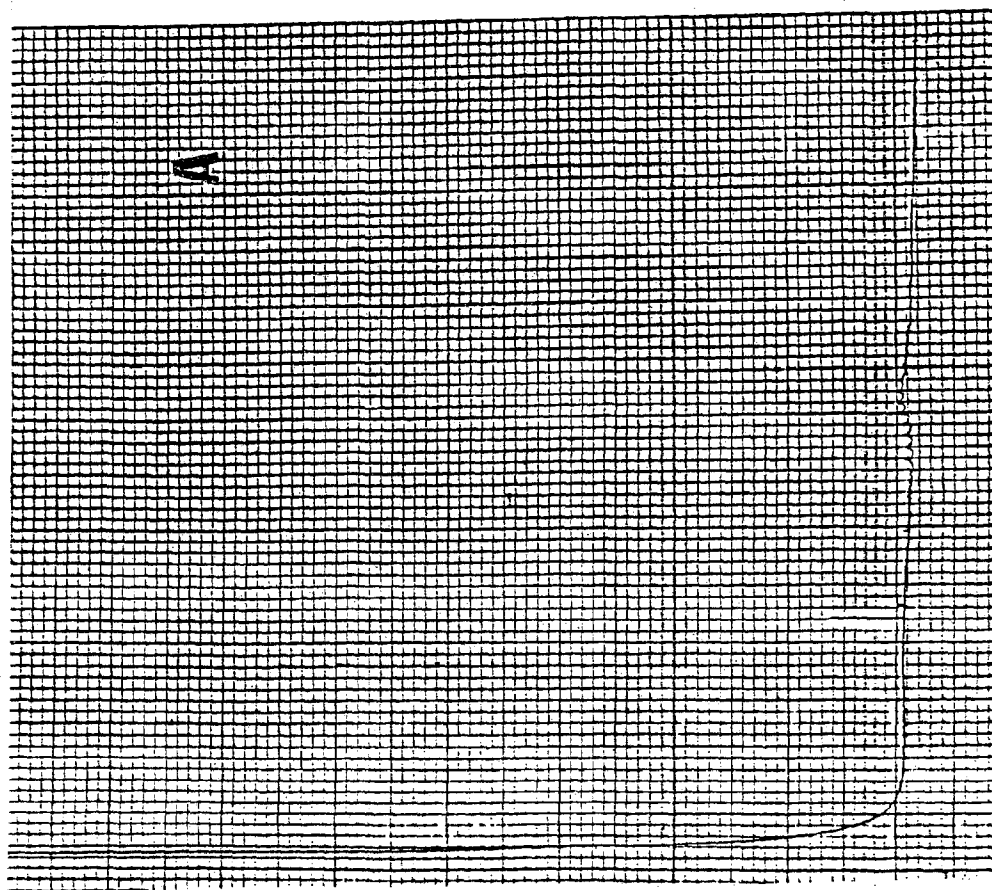


Figure 7. A. Gas chromatograms of the aliphatic hydrocarbons in control oysters from the St. Mary's River. B. Gas chromatograms of the aromatic hydrocarbons in control oysters from the St. Mary's River. Peaks in the normal alkane homologous series from $n-C_{14}$ to $n-C_{32}$ are labeled by carbon number.

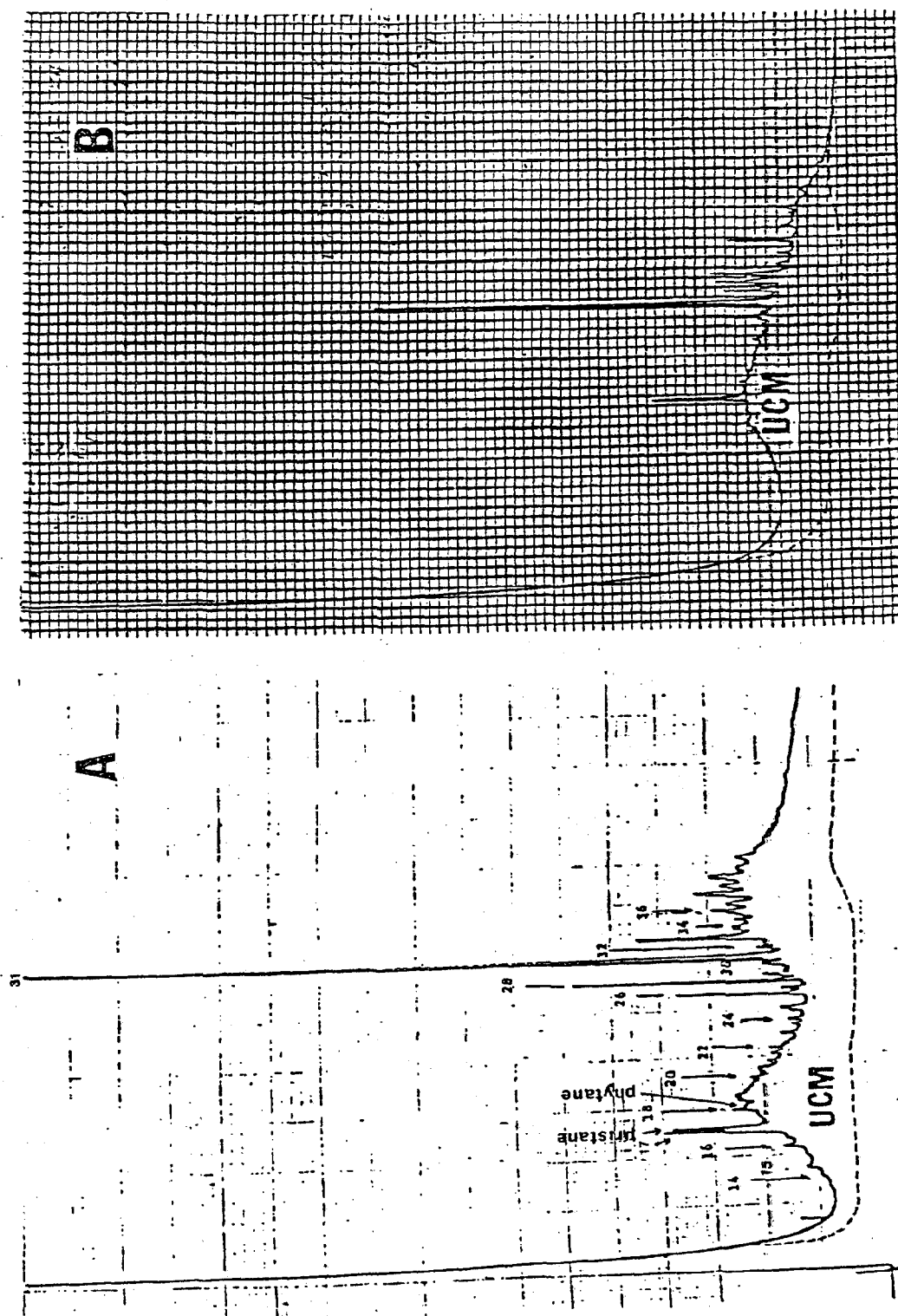


Figure 8. A. Gas Chromatogram of aliphatic hydrocarbons in oyster monitors from the Bloody Point Bar station.
B. Gas chromatogram of aromatic hydrocarbons in oyster monitors from the Bloody Point Bar station.
Peaks in the normal alkane homologous series from $n-C_{14}$ to $n-C_{32}$ are labeled by carbon number.

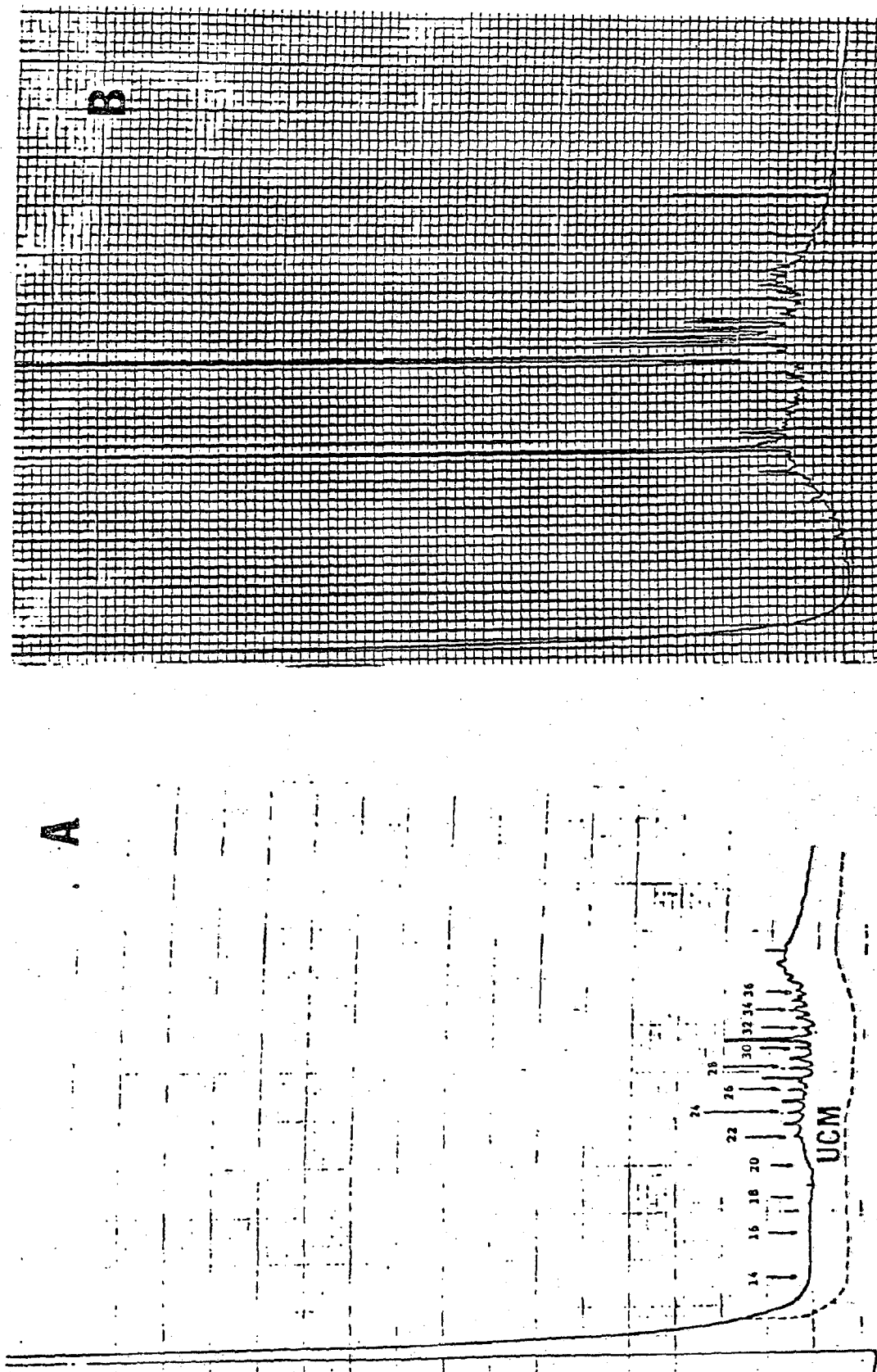


Figure 9. A. Gas chromatogram of aliphatic hydrocarbons in oyster monitors from Tolley Point station.
B. Gas chromatogram of aromatic hydrocarbons in oyster monitors from the Tolley Point station.
Peaks in the normal alkane homologous series from $n-C_{14}$ to $n-C_{32}$ are labeled by carbon number.

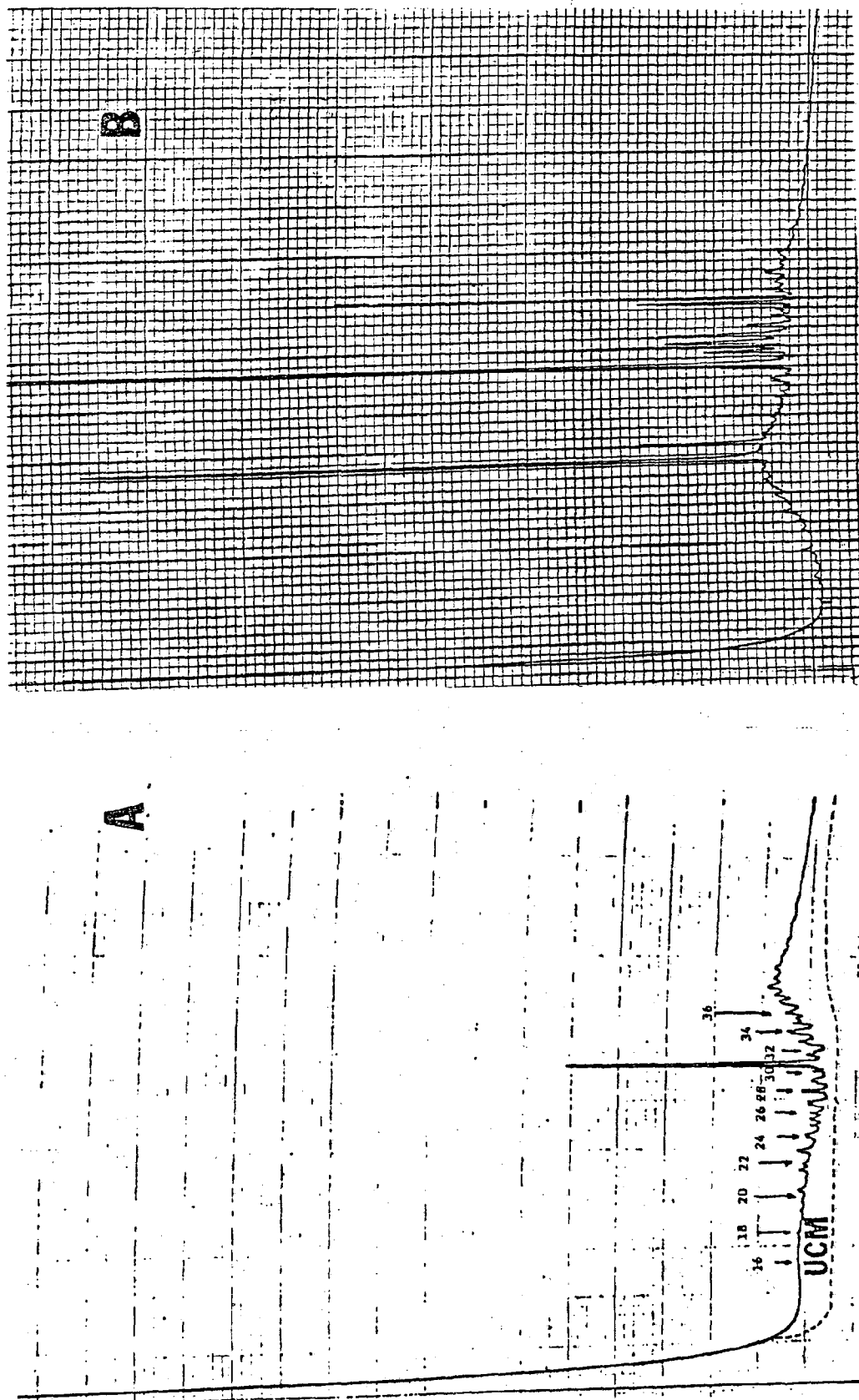


Figure 10. A. Gas chromatogram of aliphatic hydrocarbons in oyster monitors from the Brickhouse Bar station. B. Gas chromatogram of aromatic hydrocarbons in oyster monitors from the Brickhouse Bar station. Peaks in the normal alkane homologous series from $n-C_{14}$ to $n-C_{32}$ are labeled by carbon number. Contam. is an unidentified contaminant that coelutes with $n-C_{28}$.

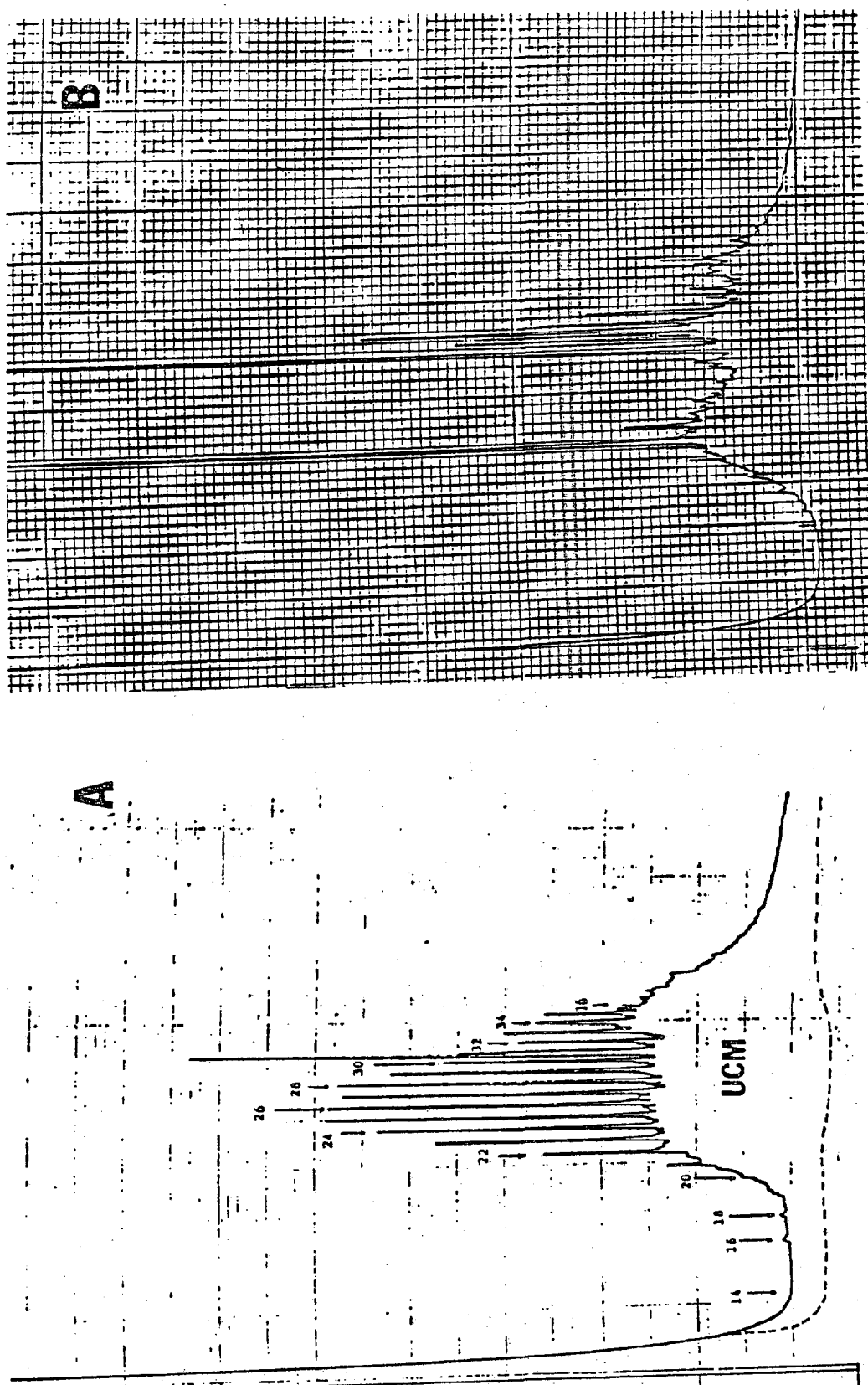


Figure 11. A. Gas chromatogram of aliphatic hydrocarbons in oyster monitors from the Patapsco River station.
B. Gas chromatogram of aromatic hydrocarbons in oyster monitors from the Patapsco River station.

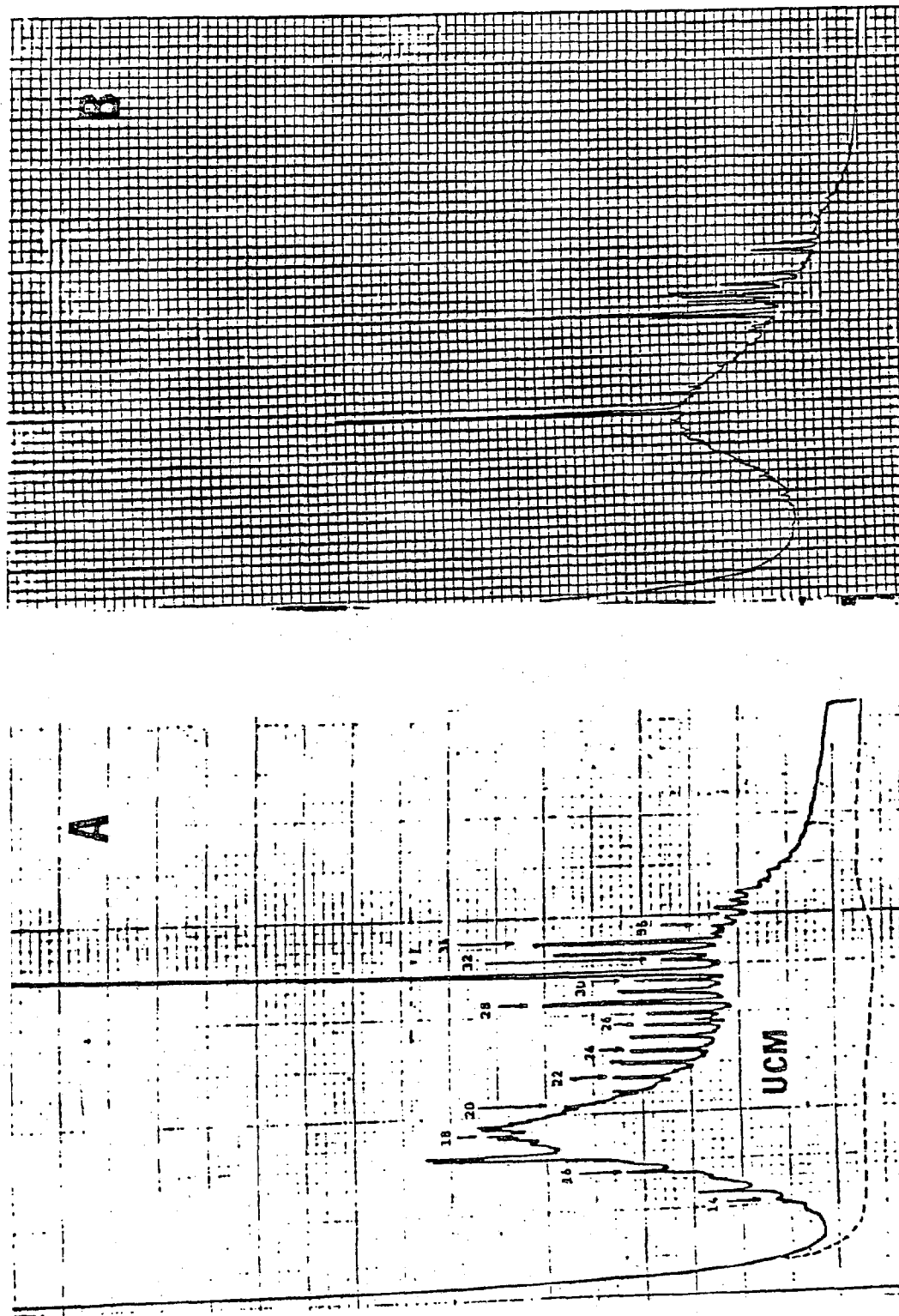


Figure 12. Gas chromatograms of aliphatic hydrocarbons in oyster gill tissue from:
 A. the second retrieval at Brickhouse Bar station; B. the first retrieval at
 Cedarhurst station; C. the third retrieval at Brickhouse Bar station.

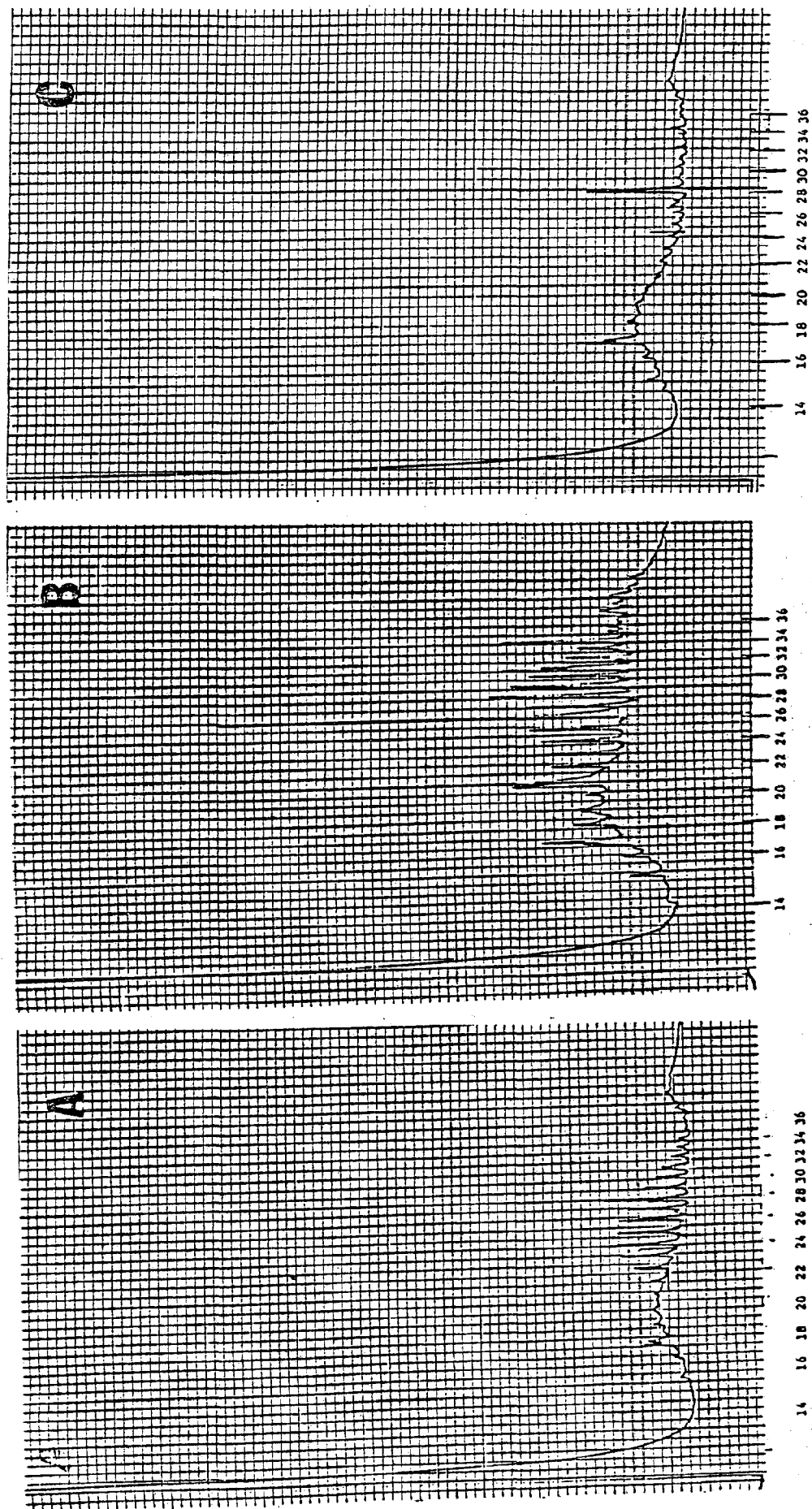


Figure 13. Gas chromatograms of aliphatic hydrocarbons in oyster gill tissue from: A. the third retrieval at Thomas Point Shoal station; B. the fourth retrieval at Thomas Point Shoal station.

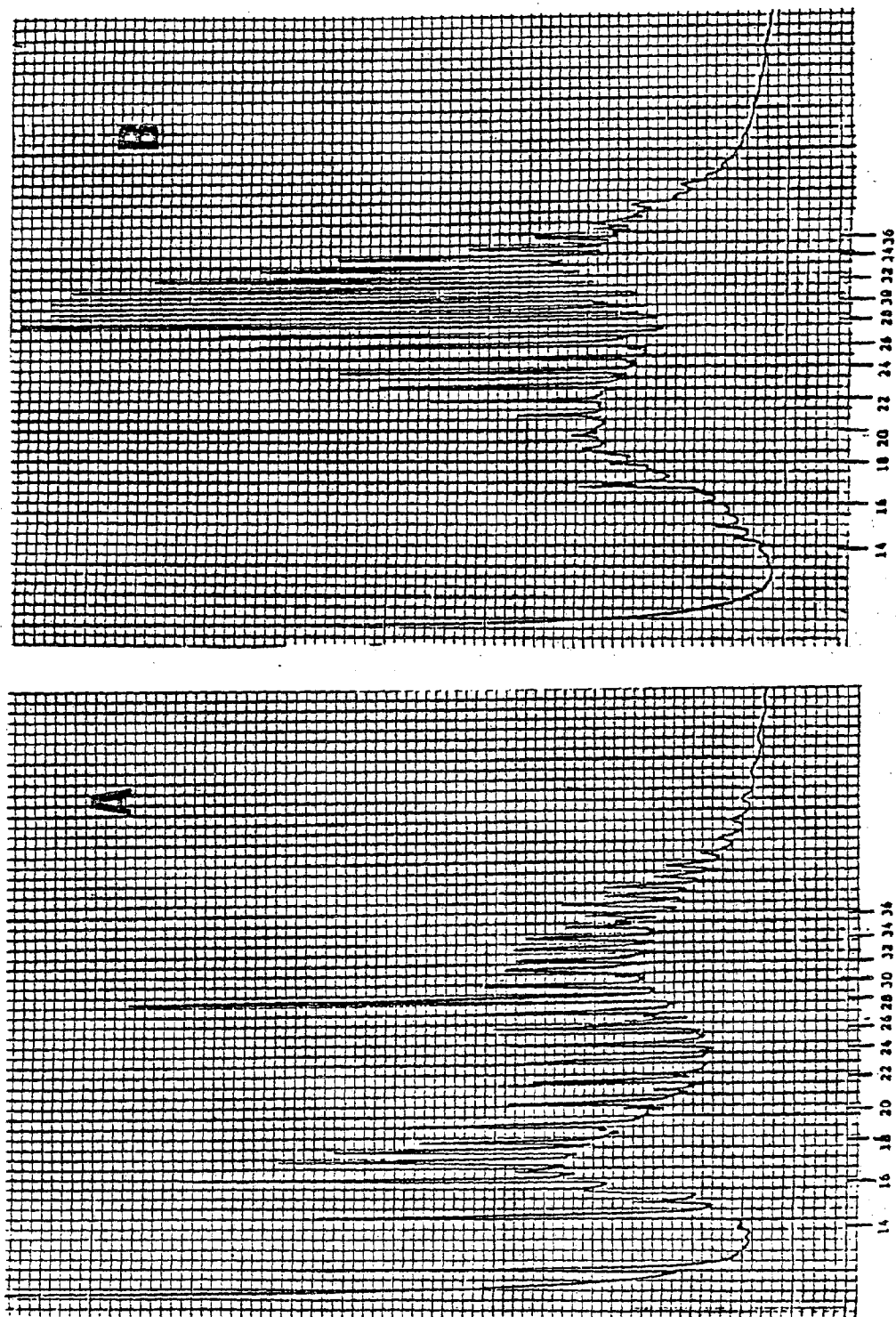


Figure 14. Gas chromatograms of aliphatic hydrocarbons in oyster gill tissue from:
A. the fourth retrieval at Brickhouse Bar station; B. the third retrieval
at Bloody Point Bar station.

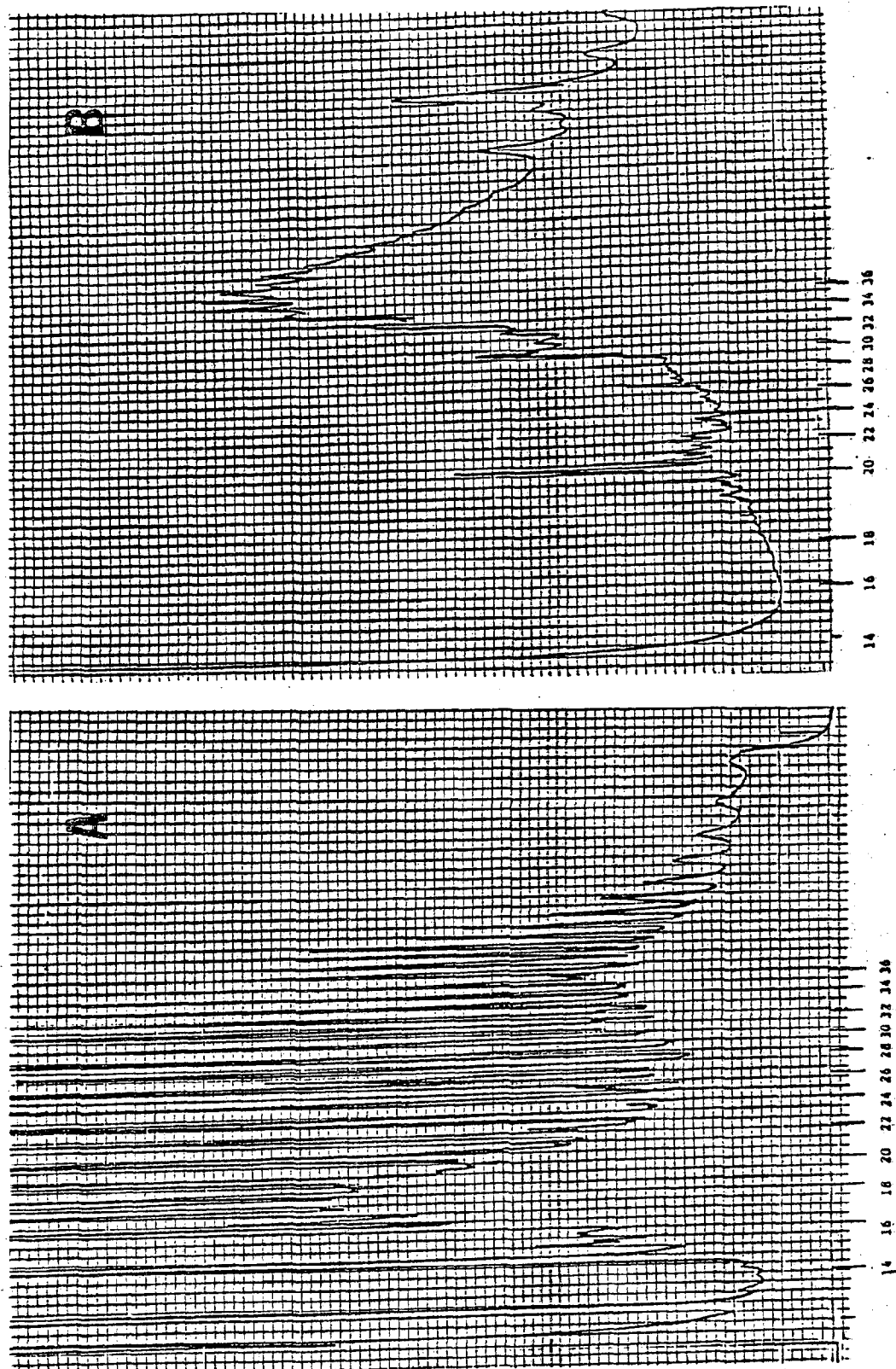


Figure 15. Gas chromatogram of aliphatic hydrocarbons in oyster gill tissue from: A. the fourth retrieval at Cedarhurst station; B. the third retrieval at Hackett Point station.

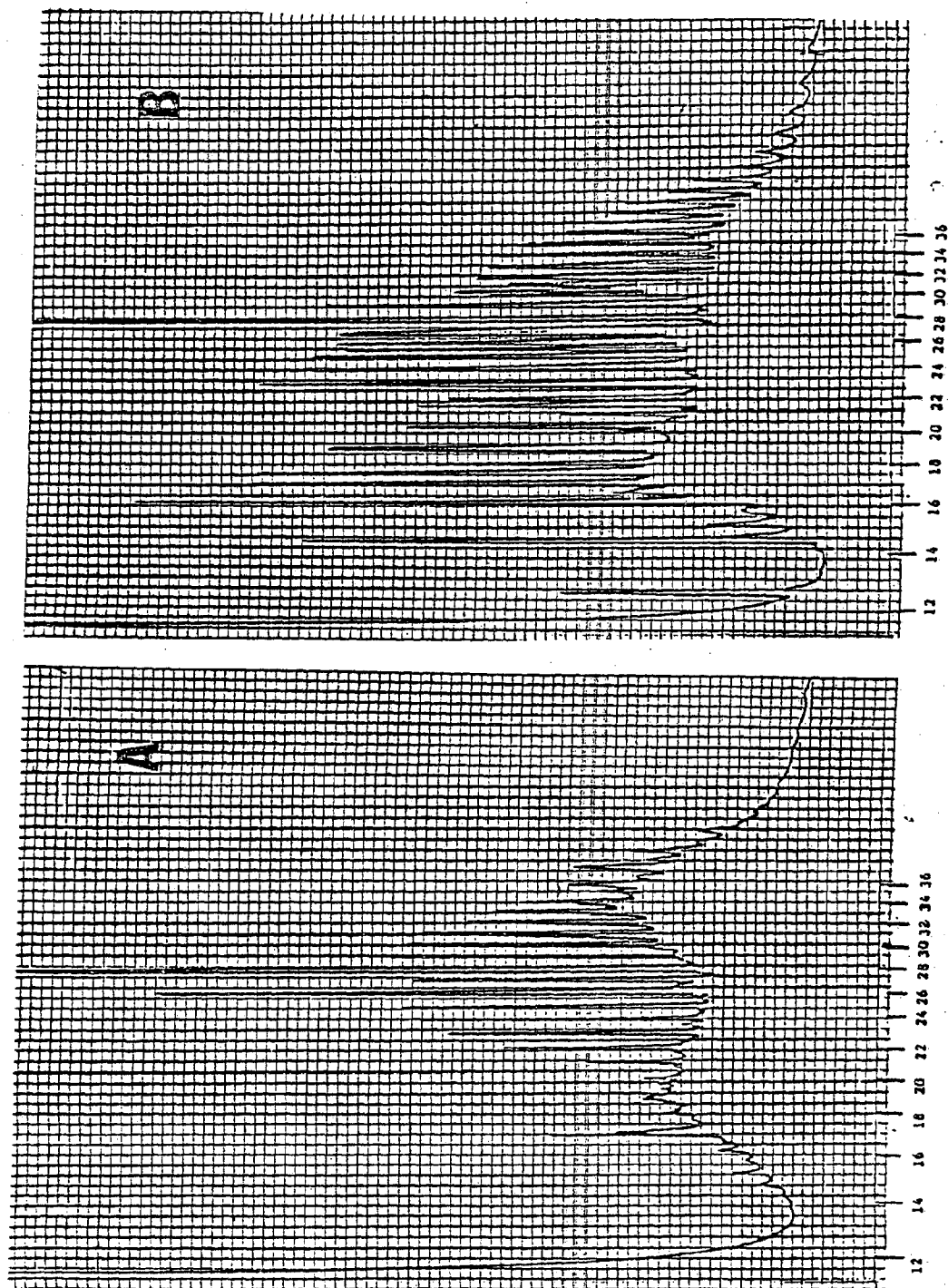
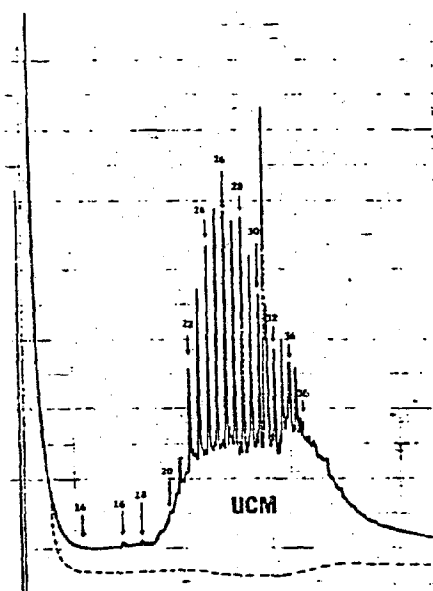
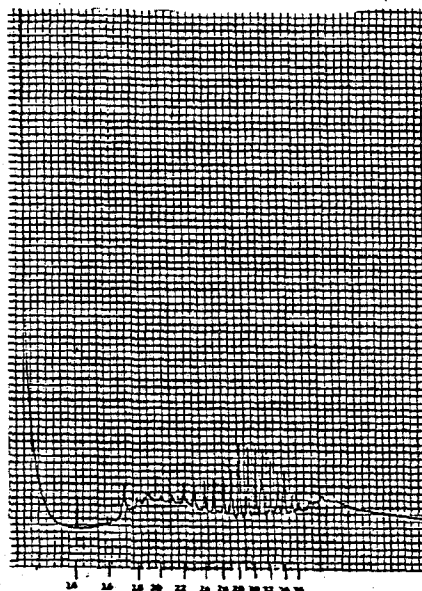


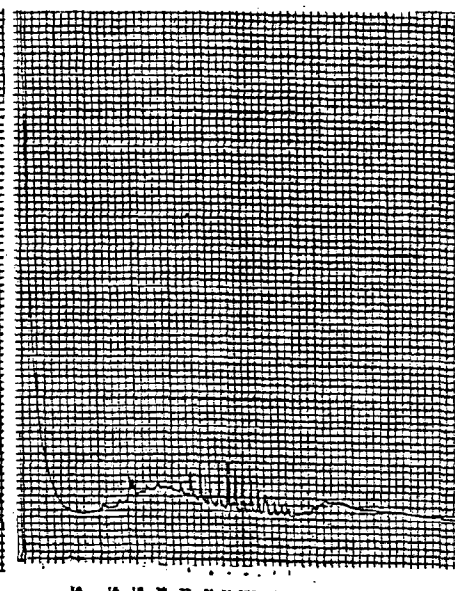
Figure 16. A. Gas chromatogram of aliphatic hydrocarbons in oyster whole body tissue from the Brickhouse Bar station. Gas chromatograms of aliphatic hydrocarbons in oyster gill tissue from the first through the fourth retrievals at the Brickhouse Bar station (B-E, respectively).



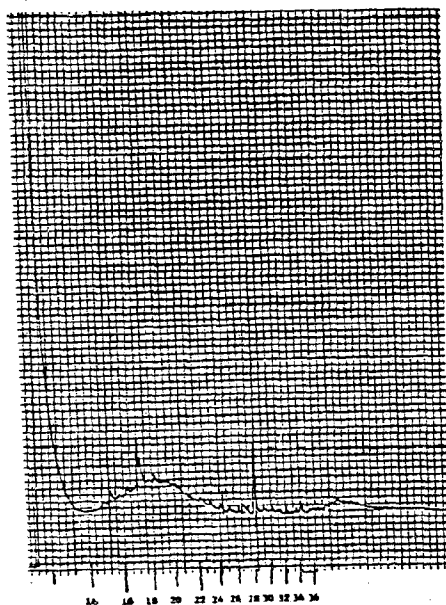
A



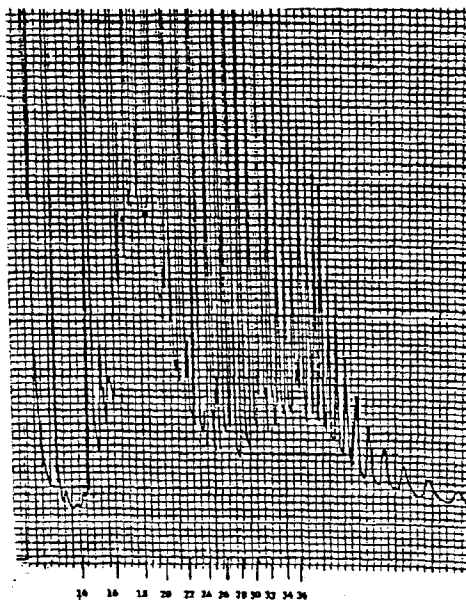
B



C



D



E

Figure 17. Gas chromatogram of aliphatic hydrocarbons in: A. oyster whole body tissues from the Bloody Point Bar station; B. oyster gill tissue from the first retrieval at Bloody Point Bar station.

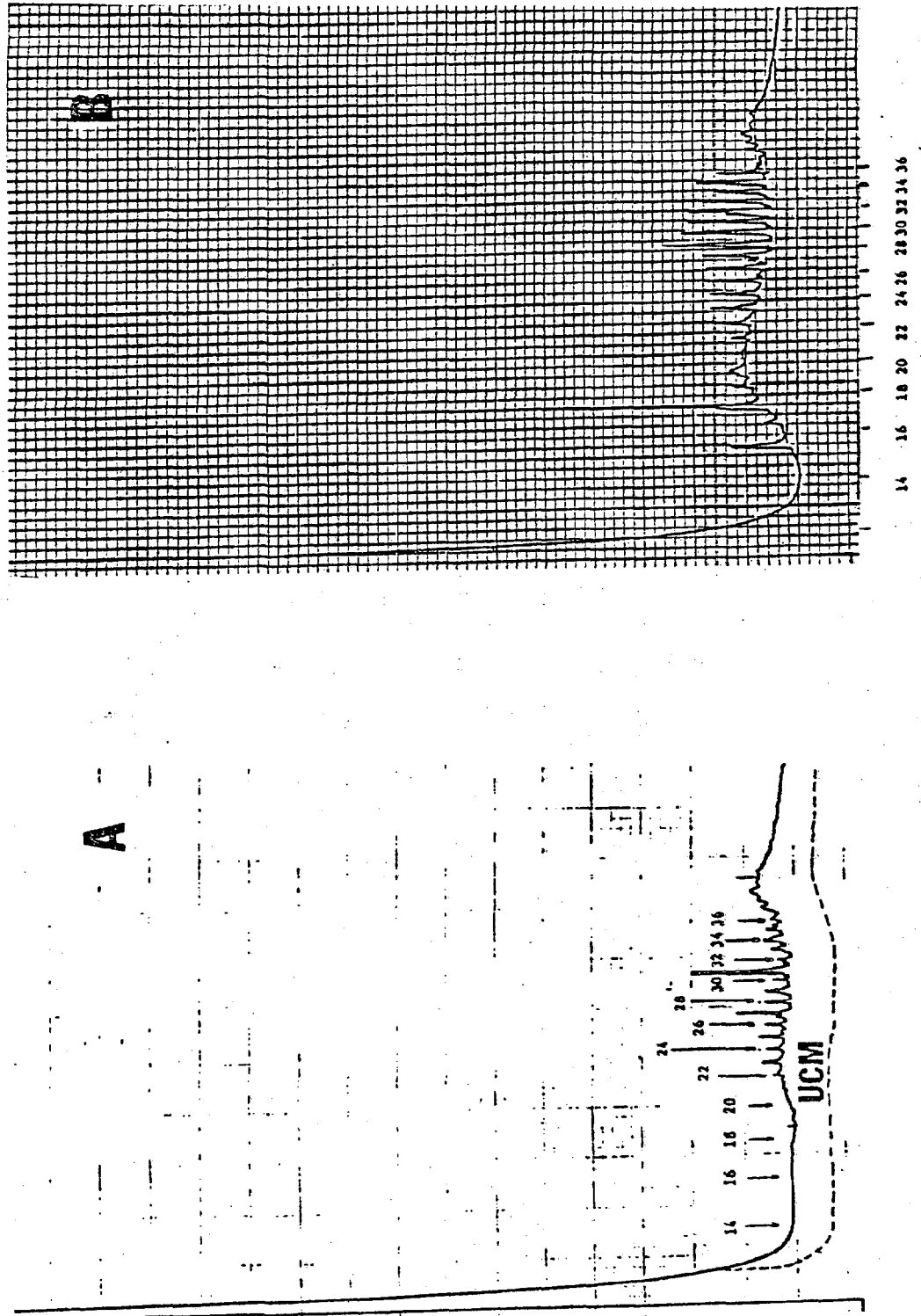


Figure 18. Gas chromatograms of the hydrocarbon content of sediments collected at A. the Patapsco River station, and B. the Brickhouse Bar station.

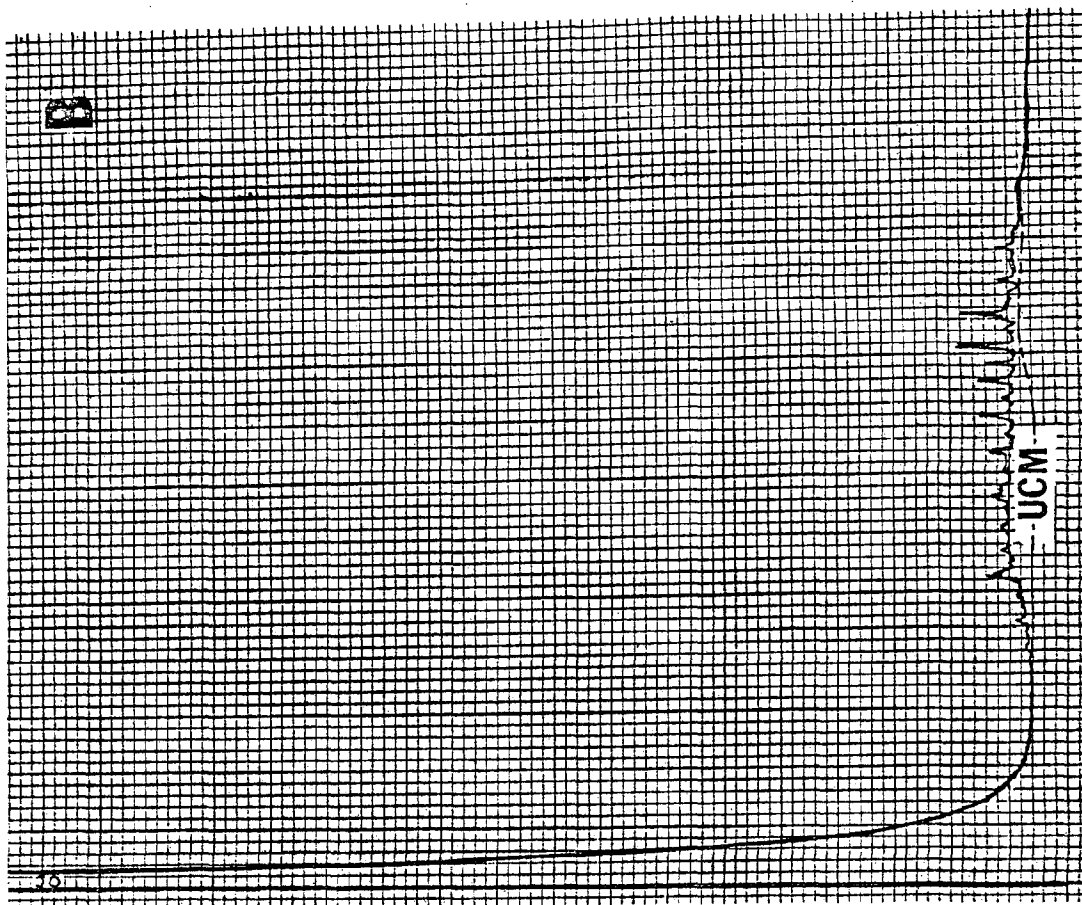
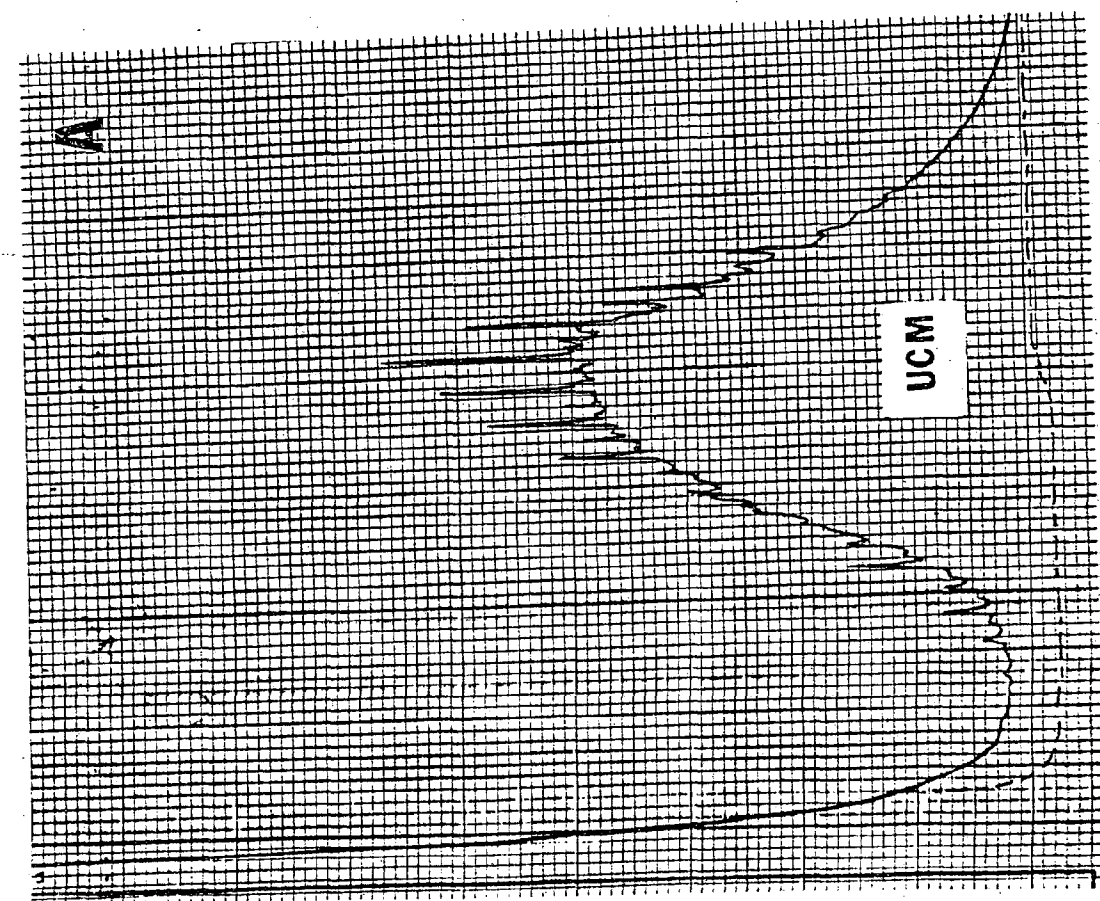


Figure 19. Gas chromatogram of the combined (total) aliphatic and aromatic hydrocarbons in fresh A. Southern Louisiana crude oil, B. No. 6 fuel oil, and C. No. 2 fuel oil. D. Gas chromatogram of n-alkane standard containing n-C₁₂ to n-C₃₆ homologous series.

